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(54) Title: COMPOSITIONS AND METHODS RELATING TO COLON SPECIFIC GENES AND PROTEINS

(57) Abstract: The present invention relates to newly identified nucleic acids and polypeptides present in normal and neoplastic colon cells, including fragments, variants and derivatives of the nucleic acids and polypeptides. The present invention also relates to antibodies to the polypeptides of the invention, as well as agonists and antagonists of the polypeptides of the invention. The invention also relates to compositions comprising the nucleic acids, polypeptides, antibodies, variants, derivatives, agonists and antagonists of the invention and methods for the use of these compositions. These uses include identifying, diagnosing, monitoring, staging, imaging and treating colon cancer and non cancerous disease states in colon tissue, identifying colon tissue, monitoring and identifying and/or designing agonists and antagonists of polypeptides of the invention. The uses also include gene therapy, production of transgenic animals and cells, and production of engineered colon tissue for treatment and research.

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## COMPOSITIONS AND METHODS RELATING TO COLON SPECIFIC GENES AND PROTEINS

This application claims the benefit of priority from U.S. Provisional Application  
5 Serial No. 60/244,758 filed October 31, 2000, which is herein incorporated by reference  
in its entirety.

### FIELD OF THE INVENTION

The present invention relates to newly identified nucleic acid molecules and  
10 polypeptides present in normal and neoplastic colon cells, including fragments, variants  
and derivatives of the nucleic acids and polypeptides. The present invention also relates  
to antibodies to the polypeptides of the invention, as well as agonists and antagonists of  
the polypeptides of the invention. The invention also relates to compositions comprising  
the nucleic acids, polypeptides, antibodies, variants, derivatives, agonists and antagonists  
15 of the invention and methods for the use of these compositions. These uses include  
identifying, diagnosing, monitoring, staging, imaging and treating colon cancer and non-  
cancerous disease states in colon tissue, identifying colon tissue and monitoring and  
identifying and/or designing agonists and antagonists of polypeptides of the invention.  
The uses also include gene therapy, production of transgenic animals and cells, and  
20 production of engineered colon tissue for treatment and research.

### BACKGROUND OF THE INVENTION

Colorectal cancer is the second most common cause of cancer death in the United  
States and the third most prevalent cancer in both men and women. M. L. Davila & A.  
D. Davila, *Screening for Colon and Rectal Cancer*, in Colon and Rectal Cancer 47 (Peter  
25 S. Edelstein ed., 2000). Approximately 100,000 patients every year suffer from colon  
cancer and approximately half that number die of the disease. Hannah-Ngoc Ha & Bard  
C. Cosman, *Treatment of Colon Cancer*, in Colon and Rectal Cancer 157 (Peter S.  
Edelstein ed., 2000). Nearly all cases of colorectal cancer arise from adenomatous  
polyps, some of which mature into large polyps, undergo abnormal growth and  
30 development, and ultimately progress into cancer. Davila & Davila, *supra* at 55-56.  
This progression would appear to take at least 10 years in most patients, rendering it a

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readily treatable form of cancer if diagnosed early, when the cancer is localized. *Id.* at 56; Walter J. Burdette, Cancer: Etiology, Diagnosis, and Treatment 125 (1998).

Although our understanding of the etiology of colon cancer is undergoing continual refinement, extensive research in this area points to a combination of factors, including age, hereditary and nonhereditary conditions, and environmental/dietary factors. Age is a key risk factor in the development of colorectal cancer, Davila & Davila, *supra* at 48, with men and women over 40 years of age become increasingly susceptible to that cancer, Burdette, *supra* at 126. Incidence rates increase considerably in each subsequent decade of life. Davila et al., *supra* at 48. A number of hereditary and nonhereditary conditions have also been linked to a heightened risk of developing colorectal cancer, including familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancer (Lynch syndrome or HNPCC), a personal and/or family history of colorectal cancer or adenomatous polyps, inflammatory bowel disease, diabetes mellitus, and obesity. *Id.* at 47; Henry T. Lynch & Jane F. Lynch, *Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndromes)*, in Colon and Rectal Cancer 67-68 (Peter S. Edelstein ed., 2000).

In the case of FAP, the tumor suppressor gene APC (adenomatous polyposis coli), located at 5q21, has been either mutationally inactivated or deleted. Alberts et al., Molecular Biology of the Cell 1288 (3d ed. 1994). The APC protein plays a role in a number of functions, including cell adhesion, apoptosis, and repression of the *c-myc* oncogene. N. R. Hall & R. D. Madoff, *Genetics and the Polyp-Cancer Sequence*, Colon and Rectal Cancer 8 (Peter S. Edelstein, ed., 2000). Of those patients with colorectal cancer who have normal APC genes, over 65% have such mutations in the cancer cells but not in other tissues. Alberts et al., *supra* at 1288. In the case of HNPCC, patients manifest abnormalities in the tumor suppressor gene HNPCC, but only about 15% of tumors contain the mutated gene. *Id.* A host of other genes have also been implicated in colorectal cancer, including the *K-ras*, *N-ras*, *H-ras* and *c-myc* oncogenes, and the tumor suppressor genes *DCC* (deleted in colon carcinoma) and *p53*. Hall & Madoff, *supra* at 8-9; Alberts et al., *supra* at 1288.

Environmental/dietary factors associated with an increased risk of colorectal cancer include a high fat diet, intake of high dietary red meat, and sedentary lifestyle. Davila & Davila, *supra* at 47; Reddy, B. S., *Prev. Med.* 16(4): 460-7 (1987).

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Conversely, environmental/dietary factors associated with a reduced risk of colorectal cancer include a diet high in fiber, folic acid, calcium, and hormone-replacement therapy in post-menopausal women. Davila & Davila, *supra* at 50-55. The effect of antioxidants in reducing the risk of colon cancer is unclear. *Id.* at 53.

5        Because colon cancer is highly treatable when detected at an early, localized stage, screening should be a part of routine care for all adults starting at age 50, especially those with first-degree relatives with colorectal cancer. One major advantage of colorectal cancer screening over its counterparts in other types of cancer is its ability to not only detect precancerous lesions, but to remove them as well. Davila & Davila,  
10    *supra* at 56. The key colorectal cancer screening tests in use today are fecal occult blood test, sigmoidoscopy, colonoscopy, double-contrast barium enema, and the carcinoembryonic antigen (CEA) test. *Id.*; Burdette, *supra* at 125.

      The fecal occult blood test (FOBT) screens for colorectal cancer by detecting the amount of blood in the stool, the premise being that neoplastic tissue, particularly  
15    malignant tissue, bleeds more than typical mucosa, with the amount of bleeding increasing with polyp size and cancer stage. Davila & Davila, *supra* at 56-57. While effective at detecting early stage tumors, FOBT is unable to detect adenomatous polyps (pre-malignant lesions), and, depending on the contents of the fecal sample, is subject to rendering false positives. *Id.* at 56-59. Sigmoidoscopy and colonoscopy, by contrast,  
20    allow direct visualization of the bowel, and enable one to detect, biopsy, and remove adenomatous polyps. *Id.* at 59-60, 61. Despite the advantages of these procedures, there are accompanying downsides: sigmoidoscopy, by definition, is limited to the sigmoid colon and below, colonoscopy is a relatively expensive procedure, and both share the risk of possible bowel perforation and hemorrhaging. *Id.* at 59-60. Double-contrast barium  
25    enema (DCBE) enables detection of lesions better than FOBT, and almost as well as colonoscopy, but it may be limited in evaluating the winding rectosigmoid region. *Id.* at 60. The CEA blood test, which involves screening the blood for carcinoembryonic antigen, shares the downside of FOBT, in that it is of limited utility in detecting colorectal cancer at an early stage. Burdette, *supra* at 125.

30        Once colon cancer has been diagnosed, treatment decisions are typically made in reference to the stage of cancer progression. A number of techniques are employed to stage the cancer (some of which are also used to screen for colon cancer), including



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pathologic examination of resected colon, sigmoidoscopy, colonoscopy, and various imaging techniques. AJCC Cancer Staging Handbook 84 (Irvin D. Fleming et al. eds., 5<sup>th</sup> ed. 1998); Montgomery, R. C. and Ridge, J.A., *Semin. Surg. Oncol.* 15(3): 143-150 (1998). Moreover, chest films, liver functionality tests, and liver scans are employed to  
5 determine the extent of metastasis. Fleming et al. eds., *supra* at 84. While computerized tomography and magnetic resonance imaging are useful in staging colorectal cancer in its later stages, both have unacceptably low staging accuracy for identifying early stages of the disease, due to the difficulty that both methods have in (1) revealing the depth of bowel wall tumor infiltration and (2) diagnosing malignant adenopathy. Thoeni, R. F.,  
10 *Radiol. Clin. N. Am.* 35(2): 457-85 (1997). Rather, techniques such as transrectal ultrasound (TRUS) are preferred in this context, although this technique is inaccurate with respect to detecting small lymph nodes that may contain metastases. David Blumberg & Frank G. Opelka, *Neoadjuvant and Adjuvant Therapy for Adenocarcinoma of the Rectum*, in Colon and Rectal Cancer 316 (Peter S. Edelstein ed., 2000).

15         Several classification systems have been devised to stage the extent of colorectal cancer, including the Dukes' system and the more detailed International Union against Cancer-American Joint Committee on Cancer TNM staging system, which is considered by many in the field to be a more useful staging system. Burdette, *supra* at 126-27. The TNM system, which is used for either clinical or pathological staging, is divided into four  
20 stages, each of which evaluates the extent of cancer growth with respect to primary tumor (T), regional lymph nodes (N), and distant metastasis (M). Fleming et al. eds., *supra* at 84-85. The system focuses on the extent of tumor invasion into the intestinal wall, invasion of adjacent structures, the number of regional lymph nodes that have been affected, and whether distant metastasis has occurred. *Id.* at 81.

25         Stage 0 is characterized by *in situ* carcinoma (Tis), in which the cancer cells are located inside the glandular basement membrane (intraepithelial) or lamina propria (intramucosal). *Id.* at 84-85; Burdette, *supra* at 127. In this stage, the cancer has not spread to the regional lymph nodes (N0), and there is no distant metastasis (M0). Fleming et al. eds., *supra* at 85; Burdette, *supra* at 127. In stage I, there is still no spread  
30 of the cancer to the regional lymph nodes and no distant metastasis, but the tumor has invaded the submucosa (T1) or has progressed further to invade the muscularis propria (T2). Fleming et al. eds., *supra* at 84-85; Burdette, *supra* at 127. Stage II also involves

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no spread of the cancer to the regional lymph nodes and no distant metastasis, but the tumor has invaded the subserosa, or the nonperitonealized pericolic or perirectal tissues (T3), or has progressed to invade other organs or structures, and/or has perforated the visceral peritoneum (T4). *Id.* Stage 3 is characterized by any of the T substages, no distant metastasis, and either metastasis in 1 to 3 regional lymph nodes (N1) or metastasis in four or more regional lymph nodes (N2). Fleming et al. eds., *supra* at 85; Burdette, *supra* at 127. Lastly, stage 4 involves any of the T or N substages, as well as distant metastasis. *Id.*

Currently, pathological staging of colon cancer is preferable over clinical staging as pathological staging provides a more accurate prognosis. Pathological staging typically involves examination of the resected colon section, along with surgical examination of the abdominal cavity. Fleming et al. eds., *supra* at 84. Clinical staging would be a preferred method of staging were it at least as accurate as pathological staging, as it does not depend on the invasive procedures of its counterpart.

Turning to the treatment of colorectal cancer, surgical resection results in a cure for roughly 50% of patients. Burdette, *supra* at 125. Irradiation is used both preoperatively and postoperatively in treating colorectal cancer. *Id.* at 125, 132-33. Chemotherapeutic agents, particularly 5-fluorouracil, are also powerful weapons in treating colorectal cancer. *Id.* at 125, 133. Other agents include irinotecan and floxuridine, cisplatin, levamisole, methotrexate, interferon-alpha, and leucovorin. *Id.* at 133. Nonetheless, thirty to forty percent of patients will develop a recurrence of colon cancer following surgical resection. Wayne De Vos, *Follow-up After Treatment of Colon Cancer, Colon and Rectal Cancer* 225 (Peter S. Edelstein ed., 2000), which in many patients is the ultimate cause of death. Accordingly, colon cancer patients must be closely monitored to determine response to therapy and to detect persistent or recurrent disease and metastasis.

From the foregoing, it is clear that procedures used for detecting, diagnosing, monitoring, staging, prognosticating, and preventing the recurrence of colorectal cancer are of critical importance to the outcome of the patient. Moreover, current procedures, while helpful in each of these analyses, are limited by their specificity, sensitivity, invasiveness, and/or their cost. As such, highly specific and sensitive procedures that

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would operate by way of detecting novel markers in cells, tissues, or bodily fluids, with minimal invasiveness and at a reasonable cost, would be highly desirable.

Accordingly, there is a great need for more sensitive and accurate methods for predicting whether a person is likely to develop colorectal cancer, for diagnosing

5 colorectal cancer, for monitoring the progression of the disease, for staging the colorectal cancer, for determining whether the colorectal cancer has metastasized, and for imaging the colorectal cancer. There is also a need for better treatment of colorectal cancer.

### SUMMARY OF THE INVENTION

10 The present invention solves these and other needs in the art by providing nucleic acid molecules and polypeptides as well as antibodies, agonists and antagonists, thereto that may be used to identify, diagnose, monitor, stage, image and treat colon cancer and non-cancerous disease states in colon; identify and monitor colon tissue; and identify and design agonists and antagonists of polypeptides of the invention. The invention also  
15 provides gene therapy, methods for producing transgenic animals and cells, and methods for producing engineered colon tissue for treatment and research.

Accordingly, one object of the invention is to provide nucleic acid molecules that are specific to colon cells and/or colon tissue. These colon specific nucleic acids (CSNAs) may be a naturally-occurring cDNA, genomic DNA, RNA, or a fragment of  
20 one of these nucleic acids, or may be a non-naturally-occurring nucleic acid molecule. If the CSNA is genomic DNA, then the CSNA is a colon specific gene (CSG). In a preferred embodiment, the nucleic acid molecule encodes a polypeptide that is specific to colon. In a more preferred embodiment, the nucleic acid molecule encodes a polypeptide that comprises an amino acid sequence of SEQ ID NO: 101 through 176. In another  
25 highly preferred embodiment, the nucleic acid molecule comprises a nucleic acid sequence of SEQ ID NO: 1 through 100. By nucleic acid molecule, it is also meant to be inclusive of sequences that selectively hybridize or exhibit substantial sequence similarity to a nucleic acid molecule encoding a CSP, or that selectively hybridize or exhibit substantial sequence similarity to a CSNA, as well as allelic variants of a nucleic  
30 acid molecule encoding a CSP, and allelic variants of a CSNA. Nucleic acid molecules comprising a part of a nucleic acid sequence that encodes a CSP or that comprises a part of a nucleic acid sequence of a CSNA are also provided.

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A related object of the present invention is to provide a nucleic acid molecule comprising one or more expression control sequences controlling the transcription and/or translation of all or a part of a CSNA. In a preferred embodiment, the nucleic acid molecule comprises one or more expression control sequences controlling the transcription and/or translation of a nucleic acid molecule that encodes all or a fragment of a CSP.

Another object of the invention is to provide vectors and/or host cells comprising a nucleic acid molecule of the instant invention. In a preferred embodiment, the nucleic acid molecule encodes all or a fragment of a CSP. In another preferred embodiment, the nucleic acid molecule comprises all or a part of a CSNA.

Another object of the invention is to provided methods for using the vectors and host cells comprising a nucleic acid molecule of the instant invention to recombinantly produce polypeptides of the invention.

Another object of the invention is to provide a polypeptide encoded by a nucleic acid molecule of the invention. In a preferred embodiment, the polypeptide is a CSP. The polypeptide may comprise either a fragment or a full-length protein as well as a mutant protein (muted), fusion protein, homologous protein or a polypeptide encoded by an allelic variant of a CSP.

Another object of the invention is to provide an antibody that specifically binds to a polypeptide of the instant invention..

Another object of the invention is to provide agonists and antagonists of the nucleic acid molecules and polypeptides of the instant invention.

Another object of the invention is to provide methods for using the nucleic acid molecules to detect or amplify nucleic acid molecules that have similar or identical nucleic acid sequences compared to the nucleic acid molecules described herein. In a preferred embodiment, the invention provides methods of using the nucleic acid molecules of the invention for identifying, diagnosing, monitoring, staging, imaging and treating colon cancer and non-cancerous disease states in colon. In another preferred embodiment, the invention provides methods of using the nucleic acid molecules of the invention for identifying and/or monitoring colon tissue. The nucleic acid molecules of the instant invention may also be used in gene therapy, for producing transgenic animals and cells, and for producing engineered colon tissue for treatment and research.

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The polypeptides and/or antibodies of the instant invention may also be used to identify, diagnose, monitor, stage, image and treat colon cancer and non-cancerous disease states in colon. The invention provides methods of using the polypeptides of the invention to identify and/or monitor colon tissue, and to produce engineered colon tissue.

5 The agonists and antagonists of the instant invention may be used to treat colon cancer and non-cancerous disease states in colon and to produce engineered colon tissue.

Yet another object of the invention is to provide a computer readable means of storing the nucleic acid and amino acid sequences of the invention. The records of the computer readable means can be accessed for reading and displaying of sequences for  
10 comparison, alignment and ordering of the sequences of the invention to other sequences.

## DETAILED DESCRIPTION OF THE INVENTION

### Definitions and General Techniques

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by  
15 those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used  
20 in the art. The methods and techniques of the present invention are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. *See, e.g.,* Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press (1989) and  
25 Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 3d ed., Cold Spring Harbor Press (2001); Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates (1992, and Supplements to 2000); Ausubel *et al.*, Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology – 4<sup>th</sup> Ed., Wiley & Sons (1999); Harlow and Lane, Antibodies: A Laboratory  
30 Manual, Cold Spring Harbor Laboratory Press (1990); and Harlow and Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1999); each of which is incorporated herein by reference in its entirety.

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Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

The following terms, unless otherwise indicated, shall be understood to have the following meanings:

- 10        A "nucleic acid molecule" of this invention refers to a polymeric form of nucleotides and includes both sense and antisense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. A nucleotide refers to a ribonucleotide, deoxynucleotide or a modified form of either type of nucleotide. A "nucleic acid molecule" as used herein is synonymous with "nucleic acid" and
- 15        "polynucleotide." The term "nucleic acid molecule" usually refers to a molecule of at least 10 bases in length, unless otherwise specified. The term includes single- and double-stranded forms of DNA. In addition, a polynucleotide may include either or both naturally-occurring and modified nucleotides linked together by naturally-occurring and/or non-naturally occurring nucleotide linkages.
- 20        The nucleic acid molecules may be modified chemically or biochemically or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (*e.g.*, methyl phosphonates,
- 25        phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (*e.g.*, phosphorothioates, phosphorodithioates, etc.), pendent moieties (*e.g.*, polypeptides), intercalators (*e.g.*, acridine, psoralen, etc.), chelators, alkylators, and modified linkages (*e.g.*, alpha anomeric nucleic acids, etc.) The term "nucleic acid molecule" also includes any topological conformation, including single-stranded, double-stranded, partially
- 30        duplexed, triplexed, hairpinned, circular and padlocked conformations. Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are

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known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

A “gene” is defined as a nucleic acid molecule that comprises a nucleic acid sequence that encodes a polypeptide and the expression control sequences that surround the nucleic acid sequence that encodes the polypeptide. For instance, a gene may  
5 comprise a promoter, one or more enhancers, a nucleic acid sequence that encodes a polypeptide, downstream regulatory sequences and, possibly, other nucleic acid sequences involved in regulation of the expression of an RNA. As is well-known in the art, eukaryotic genes usually contain both exons and introns. The term “exon” refers to a  
10 nucleic acid sequence found in genomic DNA that is bioinformatically predicted and/or experimentally confirmed to contribute a contiguous sequence to a mature mRNA transcript. The term “intron” refers to a nucleic acid sequence found in genomic DNA that is predicted and/or confirmed to not contribute to a mature mRNA transcript, but rather to be “spliced out” during processing of the transcript.

15 A nucleic acid molecule or polypeptide is “derived” from a particular species if the nucleic acid molecule or polypeptide has been isolated from the particular species, or if the nucleic acid molecule or polypeptide is homologous to a nucleic acid molecule or polypeptide isolated from a particular species.

An “isolated” or “substantially pure” nucleic acid or polynucleotide (*e.g.*, an  
20 RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components that naturally accompany the native polynucleotide in its natural host cell, *e.g.*, ribosomes, polymerases, or genomic sequences with which it is naturally associated. The term embraces a nucleic acid or polynucleotide that (1) has been removed from its naturally occurring environment, (2) is not associated with all or a  
25 portion of a polynucleotide in which the “isolated polynucleotide” is found in nature, (3) is operatively linked to a polynucleotide which it is not linked to in nature, (4) does not occur in nature as part of a larger sequence or (5) includes nucleotides or internucleoside bonds that are not found in nature. The term “isolated” or “substantially pure” also can be used in reference to recombinant or cloned DNA isolates, chemically synthesized  
30 polynucleotide analogs, or polynucleotide analogs that are biologically synthesized by heterologous systems. The term “isolated nucleic acid molecule” includes nucleic acid molecules that are integrated into a host cell chromosome at a heterologous site,

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recombinant fusions of a native fragment to a heterologous sequence, recombinant vectors present as episomes or as integrated into a host cell chromosome.

A “part” of a nucleic acid molecule refers to a nucleic acid molecule that comprises a partial contiguous sequence of at least 10 bases of the reference nucleic acid molecule. Preferably, a part comprises at least 15 to 20 bases of a reference nucleic acid molecule. In theory, a nucleic acid sequence of 17 nucleotides is of sufficient length to occur at random less frequently than once in the three gigabase human genome, and thus to provide a nucleic acid probe that can uniquely identify the reference sequence in a nucleic acid mixture of genomic complexity. A preferred part is one that comprises a nucleic acid sequence that can encode at least 6 contiguous amino acid sequences (fragments of at least 18 nucleotides) because they are useful in directing the expression or synthesis of peptides that are useful in mapping the epitopes of the polypeptide encoded by the reference nucleic acid. *See, e.g., Geysen et al., Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1984); and United States Patent Nos. 4,708,871 and 5,595,915, the disclosures of which are incorporated herein by reference in their entireties. A part may also comprise at least 25, 30, 35 or 40 nucleotides of a reference nucleic acid molecule, or at least 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400 or 500 nucleotides of a reference nucleic acid molecule. A part of a nucleic acid molecule may comprise no other nucleic acid sequences. Alternatively, a part of a nucleic acid may comprise other nucleic acid sequences from other nucleic acid molecules.

The term “oligonucleotide” refers to a nucleic acid molecule generally comprising a length of 200 bases or fewer. The term often refers to single-stranded deoxyribonucleotides, but it can refer as well to single- or double-stranded ribonucleotides, RNA:DNA hybrids and double-stranded DNAs, among others. Preferably, oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19 or 20 bases in length. Other preferred oligonucleotides are 25, 30, 35, 40, 45, 50, 55 or 60 bases in length. Oligonucleotides may be single-stranded, *e.g.* for use as probes or primers, or may be double-stranded, *e.g.* for use in the construction of a mutant gene. Oligonucleotides of the invention can be either sense or antisense oligonucleotides. An oligonucleotide can be derivatized or modified as discussed above for nucleic acid molecules.



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Oligonucleotides, such as single-stranded DNA probe oligonucleotides, often are synthesized by chemical methods, such as those implemented on automated oligonucleotide synthesizers. However, oligonucleotides can be made by a variety of other methods, including *in vitro* recombinant DNA-mediated techniques and by expression of DNAs in cells and organisms. Initially, chemically synthesized DNAs typically are obtained without a 5' phosphate. The 5' ends of such oligonucleotides are not substrates for phosphodiester bond formation by ligation reactions that employ DNA ligases typically used to form recombinant DNA molecules. Where ligation of such oligonucleotides is desired, a phosphate can be added by standard techniques, such as those that employ a kinase and ATP. The 3' end of a chemically synthesized oligonucleotide generally has a free hydroxyl group and, in the presence of a ligase, such as T4 DNA ligase, readily will form a phosphodiester bond with a 5' phosphate of another polynucleotide, such as another oligonucleotide. As is well-known, this reaction can be prevented selectively, where desired, by removing the 5' phosphates of the other polynucleotide(s) prior to ligation.

The term "naturally-occurring nucleotide" referred to herein includes naturally-occurring deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "nucleotide linkages" referred to herein includes nucleotide linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. See e.g., LaPlanche *et al. Nucl. Acids Res.* 14:9081-9093 (1986); Stein *et al. Nucl. Acids Res.* 16:3209-3221 (1988); Zon *et al. Anti-Cancer Drug Design* 6:539-568 (1991); Zon *et al.*, in Eckstein (ed.) Oligonucleotides and Analogues: A Practical Approach, pp. 87-108, Oxford University Press (1991); United States Patent No. 5,151,510; Uhlmann and Peyman *Chemical Reviews* 90:543 (1990), the disclosures of which are hereby incorporated by reference.

Unless specified otherwise, the left hand end of a polynucleotide sequence in sense orientation is the 5' end and the right hand end of the sequence is the 3' end. In addition, the left hand direction of a polynucleotide sequence in sense orientation is referred to as the 5' direction, while the right hand direction of the polynucleotide sequence is referred to as the 3' direction. Further, unless otherwise indicated, each

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nucleotide sequence is set forth herein as a sequence of deoxyribonucleotides. It is intended, however, that the given sequence be interpreted as would be appropriate to the polynucleotide composition: for example, if the isolated nucleic acid is composed of RNA, the given sequence intends ribonucleotides, with uridine substituted for thymidine.

5           The term “allelic variant” refers to one of two or more alternative naturally-occurring forms of a gene, wherein each gene possesses a unique nucleotide sequence. In a preferred embodiment, different alleles of a given gene have similar or identical biological properties.

          The term “percent sequence identity” in the context of nucleic acid sequences  
10   refers to the residues in two sequences which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over a stretch of at least about nine nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36 or more nucleotides. There are a number  
15   of different algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using FASTA, Gap or Bestfit, which are programs in Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, Wisconsin. FASTA, which includes, *e.g.*, the programs FASTA2 and FASTA3, provides alignments and percent sequence identity  
20   of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183: 63-98 (1990); Pearson, *Methods Mol. Biol.* 132: 185-219 (2000); Pearson, *Methods Enzymol.* 266: 227-258 (1996); Pearson, *J. Mol. Biol.* 276: 71-84 (1998); herein incorporated by reference). Unless otherwise specified, default parameters for a particular program or algorithm are used. For instance, percent  
25   sequence identity between nucleic acid sequences can be determined using FASTA with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) or using Gap with its default parameters as provided in GCG Version 6.1, herein incorporated by reference.

          A reference to a nucleic acid sequence encompasses its complement unless  
30   otherwise specified. Thus, a reference to a nucleic acid molecule having a particular sequence should be understood to encompass its complementary strand, with its

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complementary sequence. The complementary strand is also useful, *e.g.*, for antisense therapy, hybridization probes and PCR primers.

In the molecular biology art, researchers use the terms “percent sequence identity”, “percent sequence similarity” and “percent sequence homology”

5 interchangeably. In this application, these terms shall have the same meaning with respect to nucleic acid sequences only.

The term “substantial similarity” or “substantial sequence similarity,” when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its  
10 complementary strand), there is nucleotide sequence identity in at least about 50%, more preferably 60% of the nucleotide bases, usually at least about 70%, more usually at least about 80%, preferably at least about 90%, and more preferably at least about 95-98% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed above.

15 Alternatively, substantial similarity exists when a nucleic acid or fragment thereof hybridizes to another nucleic acid, to a strand of another nucleic acid, or to the complementary strand thereof, under selective hybridization conditions. Typically, selective hybridization will occur when there is at least about 55% sequence identity, preferably at least about 65%, more preferably at least about 75%, and most preferably at  
20 least about 90% sequence identity, over a stretch of at least about 14 nucleotides, more preferably at least 17 nucleotides, even more preferably at least 20, 25, 30, 35, 40, 50, 60, 70, 80, 90 or 100 nucleotides.

Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, solvents, the base composition of the hybridizing species,  
25 length of the complementary regions, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. “Stringent hybridization conditions” and “stringent wash conditions” in the context of nucleic acid hybridization experiments depend upon a number of different physical parameters. The most important parameters include temperature of  
30 hybridization, base composition of the nucleic acids, salt concentration and length of the nucleic acid. One having ordinary skill in the art knows how to vary these parameters to achieve a particular stringency of hybridization. In general, “stringent hybridization” is

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performed at about 25°C below the thermal melting point ( $T_m$ ) for the specific DNA hybrid under a particular set of conditions. "Stringent washing" is performed at temperatures about 5°C lower than the  $T_m$  for the specific DNA hybrid under a particular set of conditions. The  $T_m$  is the temperature at which 50% of the target sequence  
5 hybridizes to a perfectly matched probe. See Sambrook (1989), *supra*, p. 9.51, hereby incorporated by reference.

The  $T_m$  for a particular DNA-DNA hybrid can be estimated by the formula:

$$T_m = 81.5^\circ\text{C} + 16.6 (\log_{10}[\text{Na}^+]) + 0.41 (\text{fraction G} + \text{C}) - 0.63 (\% \text{ formamide}) - (600/l)$$

where  $l$  is the length of the hybrid in base pairs.

10 The  $T_m$  for a particular RNA-RNA hybrid can be estimated by the formula:

$$T_m = 79.8^\circ\text{C} + 18.5 (\log_{10}[\text{Na}^+]) + 0.58 (\text{fraction G} + \text{C}) + 11.8 (\text{fraction G} + \text{C})^2 - 0.35 (\% \text{ formamide}) - (820/l).$$

The  $T_m$  for a particular RNA-DNA hybrid can be estimated by the formula:

$$T_m = 79.8^\circ\text{C} + 18.5 (\log_{10}[\text{Na}^+]) + 0.58 (\text{fraction G} + \text{C}) + 11.8 (\text{fraction G} + \text{C})^2 - 0.50$$

15 (% formamide) - (820/l).

In general, the  $T_m$  decreases by 1-1.5°C for each 1% of mismatch between two nucleic acid sequences. Thus, one having ordinary skill in the art can alter hybridization and/or washing conditions to obtain sequences that have higher or lower degrees of sequence identity to the target nucleic acid. For instance, to obtain hybridizing nucleic  
20 acids that contain up to 10% mismatch from the target nucleic acid sequence, 10-15°C would be subtracted from the calculated  $T_m$  of a perfectly matched hybrid, and then the hybridization and washing temperatures adjusted accordingly. Probe sequences may also hybridize specifically to duplex DNA under certain conditions to form triplex or other higher order DNA complexes. The preparation of such probes and suitable hybridization  
25 conditions are well-known in the art.

An example of stringent hybridization conditions for hybridization of complementary nucleic acid sequences having more than 100 complementary residues on a filter in a Southern or Northern blot or for screening a library is 50% formamide/6X SSC at 42°C for at least ten hours and preferably overnight (approximately 16 hours).  
30 Another example of stringent hybridization conditions is 6X SSC at 68°C without formamide for at least ten hours and preferably overnight. An example of moderate stringency hybridization conditions is 6X SSC at 55°C without formamide for at least ten

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hours and preferably overnight. An example of low stringency hybridization conditions for hybridization of complementary nucleic acid sequences having more than 100 complementary residues on a filter in a Southern or Northern blot or for screening a library is 6X SSC at 42°C for at least ten hours. Hybridization conditions to identify  
5 nucleic acid sequences that are similar but not identical can be identified by experimentally changing the hybridization temperature from 68°C to 42°C while keeping the salt concentration constant (6X SSC), or keeping the hybridization temperature and salt concentration constant (*e.g.* 42°C and 6X SSC) and varying the formamide concentration from 50% to 0%. Hybridization buffers may also include blocking agents  
10 to lower background. These agents are well-known in the art. *See Sambrook et al.* (1989), *supra*, pages 8.46 and 9.46-9.58, herein incorporated by reference. *See also* Ausubel (1992), *supra*, Ausubel (1999), *supra*, and Sambrook (2001), *supra*.

Wash conditions also can be altered to change stringency conditions. An example of stringent wash conditions is a 0.2x SSC wash at 65°C for 15 minutes (*see Sambrook*  
15 (1989), *supra*, for SSC buffer). Often the high stringency wash is preceded by a low stringency wash to remove excess probe. An exemplary medium stringency wash for duplex DNA of more than 100 base pairs is 1x SSC at 45°C for 15 minutes. An exemplary low stringency wash for such a duplex is 4x SSC at 40°C for 15 minutes. In general, signal-to-noise ratio of 2x or higher than that observed for an unrelated probe in  
20 the particular hybridization assay indicates detection of a specific hybridization.

As defined herein, nucleic acid molecules that do not hybridize to each other under stringent conditions are still substantially similar to one another if they encode polypeptides that are substantially identical to each other. This occurs, for example, when a nucleic acid molecule is created synthetically or recombinantly using high codon  
25 degeneracy as permitted by the redundancy of the genetic code.

Hybridization conditions for nucleic acid molecules that are shorter than 100 nucleotides in length (*e.g.*, for oligonucleotide probes) may be calculated by the formula:  
$$T_m = 81.5^{\circ}\text{C} + 16.6(\log_{10}[\text{Na}^+]) + 0.41(\text{fraction G+C}) - (600/\text{N}),$$
  
wherein N is change length and the  $[\text{Na}^+]$  is 1 M or less. *See Sambrook* (1989), *supra*, p.  
30 11.46. For hybridization of probes shorter than 100 nucleotides, hybridization is usually performed under stringent conditions (5-10°C below the  $T_m$ ) using high concentrations (0.1-1.0 pmol/ml) of probe. *Id.* at p. 11.45. Determination of hybridization using

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mismatched probes, pools of degenerate probes or “guessmers,” as well as hybridization solutions and methods for empirically determining hybridization conditions are well-known in the art. *See, e.g.*, Ausubel (1999), *supra*; Sambrook (1989), *supra*, pp. 11.45-11.57.

5           The term “digestion” or “digestion of DNA” refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes referred to herein are commercially available and their reaction conditions, cofactors and other requirements for use are known and routine to the skilled artisan. For analytical purposes, typically, 1  $\mu$ g of plasmid or DNA fragment  
10 is digested with about 2 units of enzyme in about 20  $\mu$ l of reaction buffer. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50  $\mu$ g of DNA are digested with 20 to 250 units of enzyme in proportionately larger volumes. Appropriate buffers and substrate amounts for particular restriction enzymes are described in standard laboratory manuals, such as those referenced below, and they are  
15 specified by commercial suppliers. Incubation times of about 1 hour at 37°C are ordinarily used, but conditions may vary in accordance with standard procedures, the supplier’s instructions and the particulars of the reaction. After digestion, reactions may be analyzed, and fragments may be purified by electrophoresis through an agarose or polyacrylamide gel, using well-known methods that are routine for those skilled in the  
20 art.

          The term “ligation” refers to the process of forming phosphodiester bonds between two or more polynucleotides, which most often are double-stranded DNAs. Techniques for ligation are well-known to the art and protocols for ligation are described in standard laboratory manuals and references, such as, *e.g.*, Sambrook (1989), *supra*.

25           Genome-derived “single exon probes,” are probes that comprise at least part of an exon (“reference exon”) and can hybridize detectably under high stringency conditions to transcript-derived nucleic acids that include the reference exon but do not hybridize detectably under high stringency conditions to nucleic acids that lack the reference exon. Single exon probes typically further comprise, contiguous to a first end of the exon  
30 portion, a first intronic and/or intergenic sequence that is identically contiguous to the exon in the genome, and may contain a second intronic and/or intergenic sequence that is identically contiguous to the exon in the genome. The minimum length of genome-

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derived single exon probes is defined by the requirement that the exonic portion be of sufficient length to hybridize under high stringency conditions to transcript-derived nucleic acids, as discussed above. The maximum length of genome-derived single exon probes is defined by the requirement that the probes contain portions of no more than one  
5 exon. The single exon probes may contain priming sequences not found in contiguity with the rest of the probe sequence in the genome, which priming sequences are useful for PCR and other amplification-based technologies.

The term "microarray" or "nucleic acid microarray" refers to a substrate-bound collection of plural nucleic acids, hybridization to each of the plurality of bound nucleic  
10 acids being separately detectable. The substrate can be solid or porous, planar or non-planar, unitary or distributed. Microarrays or nucleic acid microarrays include all the devices so called in Schena (ed.), DNA Microarrays: A Practical Approach (Practical Approach Series), Oxford University Press (1999); *Nature Genet.* 21(1)(suppl.):1 - 60 (1999); Schena (ed.), Microarray Biochip: Tools and Technology, Eaton Publishing  
15 Company/BioTechniques Books Division (2000). These microarrays include substrate-bound collections of plural nucleic acids in which the plurality of nucleic acids are disposed on a plurality of beads, rather than on a unitary planar substrate, as is described, *inter alia*, in Brenner *et al.*, *Proc. Natl. Acad. Sci. USA* 97(4):1665-1670 (2000).

The term "mutated" when applied to nucleic acid molecules means that  
20 nucleotides in the nucleic acid sequence of the nucleic acid molecule may be inserted, deleted or changed compared to a reference nucleic acid sequence. A single alteration may be made at a locus (a point mutation) or multiple nucleotides may be inserted, deleted or changed at a single locus. In addition, one or more alterations may be made at any number of loci within a nucleic acid sequence. In a preferred embodiment, the  
25 nucleic acid molecule comprises the wild type nucleic acid sequence encoding a CSP or is a CSNA. The nucleic acid molecule may be mutated by any method known in the art including those mutagenesis techniques described *infra*.

The term "error-prone PCR" refers to a process for performing PCR under conditions where the copying fidelity of the DNA polymerase is low, such that a high  
30 rate of point mutations is obtained along the entire length of the PCR product. *See, e.g.*, Leung *et al.*, *Technique* 1: 11-15 (1989) and Caldwell *et al.*, *PCR Methods Applic.* 2: 28-33 (1992).

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The term "oligonucleotide-directed mutagenesis" refers to a process which enables the generation of site-specific mutations in any cloned DNA segment of interest. See, e.g., Reidhaar-Olson *et al.*, *Science* 241: 53-57 (1988).

5 The term "assembly PCR" refers to a process which involves the assembly of a PCR product from a mixture of small DNA fragments. A large number of different PCR reactions occur in parallel in the same vial, with the products of one reaction priming the products of another reaction.

10 The term "sexual PCR mutagenesis" or "DNA shuffling" refers to a method of error-prone PCR coupled with forced homologous recombination between DNA molecules of different but highly related DNA sequence *in vitro*, caused by random fragmentation of the DNA molecule based on sequence similarity, followed by fixation of the crossover by primer extension in an error-prone PCR reaction. See, e.g., Stemmer, *Proc. Natl. Acad. Sci. U.S.A.* 91: 10747-10751 (1994). DNA shuffling can be carried out between several related genes ("Family shuffling").

15 The term "*in vivo* mutagenesis" refers to a process of generating random mutations in any cloned DNA of interest which involves the propagation of the DNA in a strain of bacteria such as *E. coli* that carries mutations in one or more of the DNA repair pathways. These "mutator" strains have a higher random mutation rate than that of a wild-type parent. Propagating the DNA in a mutator strain will eventually generate  
20 random mutations within the DNA.

The term "cassette mutagenesis" refers to any process for replacing a small region of a double-stranded DNA molecule with a synthetic oligonucleotide "cassette" that differs from the native sequence. The oligonucleotide often contains completely and/or partially randomized native sequence.

25 The term "recursive ensemble mutagenesis" refers to an algorithm for protein engineering (protein mutagenesis) developed to produce diverse populations of phenotypically related mutants whose members differ in amino acid sequence. This method uses a feedback mechanism to control successive rounds of combinatorial cassette mutagenesis. See, e.g., Arkin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89: 7811-7815  
30 (1992).

The term "exponential ensemble mutagenesis" refers to a process for generating combinatorial libraries with a high percentage of unique and functional mutants, wherein



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small groups of residues are randomized in parallel to identify, at each altered position, amino acids which lead to functional proteins. See, e.g., Delegrave *et al.*, *Biotechnology Research* 11: 1548-1552 (1993); Arnold, *Current Opinion in Biotechnology* 4: 450-455 (1993). Each of the references mentioned above are hereby incorporated by reference in its entirety.

“Operatively linked” expression control sequences refers to a linkage in which the expression control sequence is contiguous with the gene of interest to control the gene of interest, as well as expression control sequences that act in *trans* or at a distance to control the gene of interest.

The term “expression control sequence” as used herein refers to polynucleotide sequences which are necessary to affect the expression of coding sequences to which they are operatively linked. Expression control sequences are sequences which control the transcription, post-transcriptional events and translation of nucleic acid sequences. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (e.g., ribosome binding sites); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include the promoter, ribosomal binding site, and transcription termination sequence. The term “control sequences” is intended to include, at a minimum, all components whose presence is essential for expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

The term “vector,” as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid”, which refers to a circular double-stranded DNA loop into which additional DNA segments may be ligated. Other vectors include cosmids, bacterial artificial chromosomes (BAC) and yeast artificial chromosomes (YAC). Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Viral vectors that infect bacterial cells are referred to as bacteriophages. Certain vectors are capable of autonomous replication in a host cell into which they are

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introduced (*e.g.*, bacterial vectors having a bacterial origin of replication). Other vectors can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors  
5 are referred to herein as “recombinant expression vectors” (or simply, “expression vectors”). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, “plasmid” and “vector” may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include other forms of expression vectors that  
10 serve equivalent functions.

The term “recombinant host cell” (or simply “host cell”), as used herein, is intended to refer to a cell into which an expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding  
15 generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term “host cell” as used herein.

As used herein, the phrase “open reading frame” and the equivalent acronym “ORF” refer to that portion of a transcript-derived nucleic acid that can be translated in  
20 its entirety into a sequence of contiguous amino acids. As so defined, an ORF has length, measured in nucleotides, exactly divisible by 3. As so defined, an ORF need not encode the entirety of a natural protein.

As used herein, the phrase “ORF-encoded peptide” refers to the predicted or actual translation of an ORF.

25 As used herein, the phrase “degenerate variant” of a reference nucleic acid sequence intends all nucleic acid sequences that can be directly translated, using the standard genetic code, to provide an amino acid sequence identical to that translated from the reference nucleic acid sequence.

The term “polypeptide” encompasses both naturally-occurring and non-naturally-  
30 occurring proteins and polypeptides, polypeptide fragments and polypeptide mutants, derivatives and analogs. A polypeptide may be monomeric or polymeric. Further, a polypeptide may comprise a number of different modules within a single polypeptide

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each of which has one or more distinct activities. A preferred polypeptide in accordance with the invention comprises a CSP encoded by a nucleic acid molecule of the instant invention, as well as a fragment, mutant, analog and derivative thereof.

The term "isolated protein" or "isolated polypeptide" is a protein or polypeptide  
5 that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is free of other proteins from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be  
10 "isolated" from its naturally associated components. A polypeptide or protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well-known in the art.

A protein or polypeptide is "substantially pure," "substantially homogeneous" or "substantially purified" when at least about 60% to 75% of a sample exhibits a single  
15 species of polypeptide. The polypeptide or protein may be monomeric or multimeric. A substantially pure polypeptide or protein will typically comprise about 50%, 60%, 70%, 80% or 90% W/W of a protein sample, more usually about 95%, and preferably will be over 99% pure. Protein purity or homogeneity may be indicated by a number of means well-known in the art, such as polyacrylamide gel electrophoresis of a protein sample,  
20 followed by visualizing a single polypeptide band upon staining the gel with a stain well-known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well-known in the art for purification.

The term "polypeptide fragment" as used herein refers to a polypeptide of the instant invention that has an amino-terminal and/or carboxy-terminal deletion compared  
25 to a full-length polypeptide. In a preferred embodiment, the polypeptide fragment is a contiguous sequence in which the amino acid sequence of the fragment is identical to the corresponding positions in the naturally-occurring sequence. Fragments typically are at least 5, 6, 7, 8, 9 or 10 amino acids long, preferably at least 12, 14, 16 or 18 amino acids long, more preferably at least 20 amino acids long, more preferably at least 25, 30, 35, 40  
30 or 45, amino acids, even more preferably at least 50 or 60 amino acids long, and even more preferably at least 70 amino acids long.

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A “derivative” refers to polypeptides or fragments thereof that are substantially similar in primary structural sequence but which include, *e.g.*, *in vivo* or *in vitro* chemical and biochemical modifications that are not found in the native polypeptide. Such modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. Other modification include, *e.g.*, labeling with radionuclides, and various enzymatic modifications, as will be readily appreciated by those skilled in the art. A variety of methods for labeling polypeptides and of substituents or labels useful for such purposes are well-known in the art, and include radioactive isotopes such as  $^{125}\text{I}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ , and  $^3\text{H}$ , ligands which bind to labeled antiligands (*e.g.*, antibodies), fluorophores, chemiluminescent agents, enzymes, and antiligands which can serve as specific binding pair members for a labeled ligand. The choice of label depends on the sensitivity required, ease of conjugation with the primer, stability requirements, and available instrumentation. Methods for labeling polypeptides are well-known in the art. *See* Ausubel (1992), *supra*; Ausubel (1999), *supra*, herein incorporated by reference.

The term “fusion protein” refers to polypeptides of the instant invention comprising polypeptides or fragments coupled to heterologous amino acid sequences. Fusion proteins are useful because they can be constructed to contain two or more desired functional elements from two or more different proteins. A fusion protein comprises at least 10 contiguous amino acids from a polypeptide of interest, more preferably at least 20 or 30 amino acids, even more preferably at least 40, 50 or 60 amino acids, yet more preferably at least 75, 100 or 125 amino acids. Fusion proteins can be produced recombinantly by constructing a nucleic acid sequence which encodes the polypeptide or a fragment thereof in frame with a nucleic acid sequence encoding a

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different protein or peptide and then expressing the fusion protein. Alternatively, a fusion protein can be produced chemically by crosslinking the polypeptide or a fragment thereof to another protein.

The term "analog" refers to both polypeptide analogs and non-peptide analogs.

- 5 The term "polypeptide analog" as used herein refers to a polypeptide of the instant invention that is comprised of a segment of at least 25 amino acids that has substantial identity to a portion of an amino acid sequence but which contains non-natural amino acids or non-natural inter-residue bonds. In a preferred embodiment, the analog has the same or similar biological activity as the native polypeptide. Typically, polypeptide
- 10 analogs comprise a conservative amino acid substitution (or insertion or deletion) with respect to the naturally-occurring sequence. Analogs typically are at least 20 amino acids long, preferably at least 50 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

- The term "non-peptide analog" refers to a compound with properties that are
- 15 analogous to those of a reference polypeptide of the instant invention. A non-peptide compound may also be termed a "peptide mimetic" or a "peptidomimetic." Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to useful peptides may be used to produce an equivalent effect. Generally, peptidomimetics are structurally similar to a paradigm
- 20 polypeptide (*i.e.*, a polypeptide that has a desired biochemical property or pharmacological activity), but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: --CH<sub>2</sub>NH--, --CH<sub>2</sub>S--, --CH<sub>2</sub>-CH<sub>2</sub>--,
- CH=CH--(cis and trans), --COCH<sub>2</sub>--, --CH(OH)CH<sub>2</sub>--, and --CH<sub>2</sub>SO--, by methods well-known in the art. Systematic substitution of one or more amino acids of a
- 25 consensus sequence with a D-amino acid of the same type (*e.g.*, D-lysine in place of L-lysine) may also be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo *et al.*, *Ann. Rev. Biochem.* 61:387-418 (1992), incorporated herein by reference). For example, one may
- 30 add internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

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A "polypeptide mutant" or "mutein" refers to a polypeptide of the instant invention whose sequence contains substitutions, insertions or deletions of one or more amino acids compared to the amino acid sequence of a native or wild-type protein. A mutein may have one or more amino acid point substitutions, in which a single amino acid at a position has been changed to another amino acid, one or more insertions and/or deletions, in which one or more amino acids are inserted or deleted, respectively, in the sequence of the naturally-occurring protein, and/or truncations of the amino acid sequence at either or both the amino or carboxy termini. Further, a mutein may have the same or different biological activity as the naturally-occurring protein. For instance, a mutein may have an increased or decreased biological activity. A mutein has at least 50% sequence similarity to the wild type protein, preferred is 60% sequence similarity, more preferred is 70% sequence similarity. Even more preferred are muteins having 80%, 85% or 90% sequence similarity to the wild type protein. In an even more preferred embodiment, a mutein exhibits 95% sequence identity, even more preferably 97%, even more preferably 98% and even more preferably 99%. Sequence similarity may be measured by any common sequence analysis algorithm, such as Gap or Bestfit.

Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinity or enzymatic activity, and (5) confer or modify other physicochemical or functional properties of such analogs. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally-occurring sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. In a preferred embodiment, the amino acid substitutions are moderately conservative substitutions or conservative substitutions. In a more preferred embodiment, the amino acid substitutions are conservative substitutions. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (*e.g.*, a replacement amino acid should not tend to disrupt a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Creighton (ed.), Proteins, Structures and Molecular Principles, W. H. Freeman and Company (1984); Branden *et al.* (ed.), Introduction to Protein Structure,

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Garland Publishing (1991); Thornton *et al.*, *Nature* 354:105-106 (1991), each of which are incorporated herein by reference.

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See Golub *et al.* (eds.), Immunology - A Synthesis 2<sup>nd</sup> Ed.,  
5 Sinauer Associates (1991), which is incorporated herein by reference. Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as -, -disubstituted amino acids, N-alkyl amino acids, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4-hydroxyproline,  $\gamma$ -carboxyglutamate,  
10 -N,N,N-trimethyllysine, -N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, s-N-methylarginine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline). In the polypeptide notation used herein, the lefthand direction is the amino terminal direction and the right hand direction is the carboxy-terminal direction, in accordance with standard usage and  
15 convention.

A protein has "homology" or is "homologous" to a protein from another organism if the encoded amino acid sequence of the protein has a similar sequence to the encoded amino acid sequence of a protein of a different organism and has a similar biological activity or function. Alternatively, a protein may have homology or be homologous to  
20 another protein if the two proteins have similar amino acid sequences and have similar biological activities or functions. Although two proteins are said to be "homologous," this does not imply that there is necessarily an evolutionary relationship between the proteins. Instead, the term "homologous" is defined to mean that the two proteins have similar amino acid sequences and similar biological activities or functions. In a preferred  
25 embodiment, a homologous protein is one that exhibits 50% sequence similarity to the wild type protein, preferred is 60% sequence similarity, more preferred is 70% sequence similarity. Even more preferred are homologous proteins that exhibit 80%, 85% or 90% sequence similarity to the wild type protein. In a yet more preferred embodiment, a homologous protein exhibits 95%, 97%, 98% or 99% sequence similarity.

30 When "sequence similarity" is used in reference to proteins or peptides, it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions. In a preferred embodiment, a polypeptide that has "sequence

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similarity” comprises conservative or moderately conservative amino acid substitutions. A “conservative amino acid substitution” is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.*, Pearson, *Methods Mol. Biol.* 24: 307-31 (1994), herein incorporated by reference.

For instance, the following six groups each contain amino acids that are conservative substitutions for one another:

- 1) Serine (S), Threonine (T);
- 2) Aspartic Acid (D), Glutamic Acid (E);
- 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Alanine (A), Valine (V), and
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.*, *Science* 256: 1443-45 (1992), herein incorporated by reference. A “moderately conservative” replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as “Gap” and “Bestfit” which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutin thereof. *See, e.g.*, GCG Version 6.1. Other programs include FASTA, discussed *supra*.



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A preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn. *See, e.g., Altschul et al., J. Mol. Biol.* 215: 403-410 (1990); *Altschul et al., Nucleic Acids Res.* 25:3389-402 (1997); herein

5 incorporated by reference. Preferred parameters for blastp are:

Expectation value:	10 (default)
Filter:	seg (default)
Cost to open a gap:	11 (default)
Cost to extend a gap:	1 (default)
10 Max. alignments:	100 (default)
Word size:	11 (default)
No. of descriptions:	100 (default)
Penalty Matrix:	BLOSUM62

The length of polypeptide sequences compared for homology will generally be at  
15 least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. When searching a database containing sequences from a large number of different organisms, it is preferable to compare amino acid sequences.

Database searching using amino acid sequences can be measured by algorithms  
20 other than blastp are known in the art. For instance, polypeptide sequences can be compared using FASTA, a program in GCG Version 6.1. FASTA (*e.g., FASTA2 and FASTA3*) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (1990), *supra*; Pearson (2000), *supra*). For example, percent sequence identity between amino acid sequences can be  
25 determined using FASTA with its default or recommended parameters (a word size of 2 and the PAM250 scoring matrix), as provided in GCG Version 6.1, herein incorporated by reference.

An "antibody" refers to an intact immunoglobulin, or to an antigen-binding portion thereof that competes with the intact antibody for specific binding to a molecular  
30 species, *e.g.,* a polypeptide of the instant invention. Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen-binding portions include, *inter alia*, Fab, Fab', F(ab')<sub>2</sub>, Fv,

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dAb, and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide. An Fab fragment is a monovalent fragment consisting of the VL, VH, CL and CH1 domains; an F(ab')<sub>2</sub> fragment is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; an Fd fragment consists of the VH and CH1 domains; an Fv fragment consists of the VL and VH domains of a single arm of an antibody; and a dAb fragment consists of a VH domain. *See, e.g., Ward et al., Nature* 341: 544-546 (1989).

By "bind specifically" and "specific binding" is here intended the ability of the antibody to bind to a first molecular species in preference to binding to other molecular species with which the antibody and first molecular species are admixed. An antibody is said specifically to "recognize" a first molecular species when it can bind specifically to that first molecular species.

A single-chain antibody (scFv) is an antibody in which a VL and VH region are paired to form a monovalent molecule via a synthetic linker that enables them to be made as a single protein chain. *See, e.g., Bird et al., Science* 242: 423-426 (1988); Huston *et al., Proc. Natl. Acad. Sci. USA* 85: 5879-5883 (1988). Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites. *See e.g., Holliger et al., Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993); Poljak *et al., Structure* 2: 1121-1123 (1994). One or more CDRs may be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin. An immunoadhesin may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may incorporate the CDR(s) noncovalently. The CDRs permit the immunoadhesin to specifically bind to a particular antigen of interest. A chimeric antibody is an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies.

An antibody may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For

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instance, a naturally-occurring immunoglobulin has two identical binding sites, a single-chain antibody or Fab fragment has one binding site, while a “bispecific” or “bifunctional” antibody has two different binding sites.

An “isolated antibody” is an antibody that (1) is not associated with naturally-associated components, including other naturally-associated antibodies, that accompany it in its native state, (2) is free of other proteins from the same species, (3) is expressed by a cell from a different species, or (4) does not occur in nature. It is known that purified proteins, including purified antibodies, may be stabilized with non-naturally-associated components. The non-naturally-associated component may be a protein, such as albumin (*e.g.*, BSA) or a chemical such as polyethylene glycol (PEG).

A “neutralizing antibody” or “an inhibitory antibody” is an antibody that inhibits the activity of a polypeptide or blocks the binding of a polypeptide to a ligand that normally binds to it. An “activating antibody” is an antibody that increases the activity of a polypeptide.

The term “epitope” includes any protein determinant capable of specifically binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the dissociation constant is less than  $1\ \mu\text{M}$ , preferably less than  $100\ \text{nM}$  and most preferably less than  $10\ \text{nM}$ .

The term “patient” as used herein includes human and veterinary subjects.

Throughout this specification and claims, the word “comprise,” or variations such as “comprises” or “comprising,” will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

The term “colon specific” refers to a nucleic acid molecule or polypeptide that is expressed predominantly in the colon as compared to other tissues in the body. In a preferred embodiment, a “colon specific” nucleic acid molecule or polypeptide is expressed at a level that is 5-fold higher than any other tissue in the body. In a more preferred embodiment, the “colon specific” nucleic acid molecule or polypeptide is expressed at a level that is 10-fold higher than any other tissue in the body, more

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preferably at least 15-fold, 20-fold, 25-fold, 50-fold or 100-fold higher than any other tissue in the body. Nucleic acid molecule levels may be measured by nucleic acid hybridization, such as Northern blot hybridization, or quantitative PCR. Polypeptide levels may be measured by any method known to accurately quantitate protein levels, such as Western blot analysis.

Nucleic Acid Molecules, Regulatory Sequences, Vectors, Host Cells and Recombinant Methods of Making Polypeptides

*Nucleic Acid Molecules*

One aspect of the invention provides isolated nucleic acid molecules that are specific to the colon or to colon cells or tissue or that are derived from such nucleic acid molecules. These isolated colon specific nucleic acids (CSNAs) may comprise a cDNA, a genomic DNA, RNA, or a fragment of one of these nucleic acids, or may be a non-naturally-occurring nucleic acid molecule. In a preferred embodiment, the nucleic acid molecule encodes a polypeptide that is specific to colon, a colon-specific polypeptide (CSP). In a more preferred embodiment, the nucleic acid molecule encodes a polypeptide that comprises an amino acid sequence of SEQ ID NO: 101 through 176. In another highly preferred embodiment, the nucleic acid molecule comprises a nucleic acid sequence of SEQ ID NO: 1 through 100.

A CSNA may be derived from a human or from another animal. In a preferred embodiment, the CSNA is derived from a human or other mammal. In a more preferred embodiment, the CSNA is derived from a human or other primate. In an even more preferred embodiment, the CSNA is derived from a human.

By "nucleic acid molecule" for purposes of the present invention, it is also meant to be inclusive of nucleic acid sequences that selectively hybridize to a nucleic acid molecule encoding a CSNA or a complement thereof. The hybridizing nucleic acid molecule may or may not encode a polypeptide or may not encode a CSP. However, in a preferred embodiment, the hybridizing nucleic acid molecule encodes a CSP. In a more preferred embodiment, the invention provides a nucleic acid molecule that selectively hybridizes to a nucleic acid molecule that encodes a polypeptide comprising an amino acid sequence of SEQ ID NO: 101 through 176. In an even more preferred embodiment, the invention provides a nucleic acid molecule that selectively hybridizes to a nucleic acid molecule comprising the nucleic acid sequence of SEQ ID NO: 1 through 100.

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In a preferred embodiment, the nucleic acid molecule selectively hybridizes to a nucleic acid molecule encoding a CSP under low stringency conditions. In a more preferred embodiment, the nucleic acid molecule selectively hybridizes to a nucleic acid molecule encoding a CSP under moderate stringency conditions. In a more preferred embodiment, the nucleic acid molecule selectively hybridizes to a nucleic acid molecule encoding a CSP under high stringency conditions. In an even more preferred embodiment, the nucleic acid molecule hybridizes under low, moderate or high stringency conditions to a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of SEQ ID NO: 101 through 176. In a yet more preferred embodiment, the nucleic acid molecule hybridizes under low, moderate or high stringency conditions to a nucleic acid molecule comprising a nucleic acid sequence selected from SEQ ID NO: 1 through 100. In a preferred embodiment of the invention, the hybridizing nucleic acid molecule may be used to express recombinantly a polypeptide of the invention.

By "nucleic acid molecule" as used herein it is also meant to be inclusive of sequences that exhibits substantial sequence similarity to a nucleic acid encoding a CSP or a complement of the encoding nucleic acid molecule. In a preferred embodiment, the nucleic acid molecule exhibits substantial sequence similarity to a nucleic acid molecule encoding human CSP. In a more preferred embodiment, the nucleic acid molecule exhibits substantial sequence similarity to a nucleic acid molecule encoding a polypeptide having an amino acid sequence of SEQ ID NO: 101 through 176. In a preferred embodiment, the similar nucleic acid molecule is one that has at least 60% sequence identity with a nucleic acid molecule encoding a CSP, such as a polypeptide having an amino acid sequence of SEQ ID NO: 101 through 176, more preferably at least 70%, even more preferably at least 80% and even more preferably at least 85%. In a more preferred embodiment, the similar nucleic acid molecule is one that has at least 90% sequence identity with a nucleic acid molecule encoding a CSP, more preferably at least 95%, more preferably at least 97%, even more preferably at least 98%, and still more preferably at least 99%. In another highly preferred embodiment, the nucleic acid molecule is one that has at least 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity with a nucleic acid molecule encoding a CSP.

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In another preferred embodiment, the nucleic acid molecule exhibits substantial sequence similarity to a CSNA or its complement. In a more preferred embodiment, the nucleic acid molecule exhibits substantial sequence similarity to a nucleic acid molecule comprising a nucleic acid sequence of SEQ ID NO: 1 through 100. In a preferred  
5 embodiment, the nucleic acid molecule is one that has at least 60% sequence identity with a CSNA, such as one having a nucleic acid sequence of SEQ ID NO: 1 through 100, more preferably at least 70%, even more preferably at least 80% and even more preferably at least 85%. In a more preferred embodiment, the nucleic acid molecule is one that has at least 90% sequence identity with a CSNA, more preferably at least 95%,  
10 more preferably at least 97%, even more preferably at least 98%, and still more preferably at least 99%. In another highly preferred embodiment, the nucleic acid molecule is one that has at least 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity with a CSNA.

A nucleic acid molecule that exhibits substantial sequence similarity may be one  
15 that exhibits sequence identity over its entire length to a CSNA or to a nucleic acid molecule encoding a CSP, or may be one that is similar over only a part of its length. In this case, the part is at least 50 nucleotides of the CSNA or the nucleic acid molecule encoding a CSP, preferably at least 100 nucleotides, more preferably at least 150 or 200 nucleotides, even more preferably at least 250 or 300 nucleotides, still more preferably at  
20 least 400 or 500 nucleotides.

The substantially similar nucleic acid molecule may be a naturally-occurring one that is derived from another species, especially one derived from another primate, wherein the similar nucleic acid molecule encodes an amino acid sequence that exhibits significant sequence identity to that of SEQ ID NO: 101 through 176 or demonstrates  
25 significant sequence identity to the nucleotide sequence of SEQ ID NO: 1 through 100. The similar nucleic acid molecule may also be a naturally-occurring nucleic acid molecule from a human, when the CSNA is a member of a gene family. The similar nucleic acid molecule may also be a naturally-occurring nucleic acid molecule derived from a non-primate, mammalian species, including without limitation, domesticated  
30 species, *e.g.*, dog, cat, mouse, rat, rabbit, hamster, cow, horse and pig; and wild animals, *e.g.*, monkey, fox, lions, tigers, bears, giraffes, zebras, etc. The substantially similar nucleic acid molecule may also be a naturally-occurring nucleic acid molecule derived

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from a non-mammalian species, such as birds or reptiles. The naturally-occurring substantially similar nucleic acid molecule may be isolated directly from humans or other species. In another embodiment, the substantially similar nucleic acid molecule may be one that is experimentally produced by random mutation of a nucleic acid molecule. In another embodiment, the substantially similar nucleic acid molecule may be one that is experimentally produced by directed mutation of a CSNA. Further, the substantially similar nucleic acid molecule may or may not be a CSNA. However, in a preferred embodiment, the substantially similar nucleic acid molecule is a CSNA.

By “nucleic acid molecule” it is also meant to be inclusive of allelic variants of a CSNA or a nucleic acid encoding a CSP. For instance, single nucleotide polymorphisms (SNPs) occur frequently in eukaryotic genomes. In fact, more than 1.4 million SNPs have already identified in the human genome, International Human Genome Sequencing Consortium, *Nature* 409: 860-921 (2001). Thus, the sequence determined from one individual of a species may differ from other allelic forms present within the population. Additionally, small deletions and insertions, rather than single nucleotide polymorphisms, are not uncommon in the general population, and often do not alter the function of the protein. Further, amino acid substitutions occur frequently among natural allelic variants, and often do not substantially change protein function.

In a preferred embodiment, the nucleic acid molecule comprising an allelic variant is a variant of a gene, wherein the gene is transcribed into an mRNA that encodes a CSP. In a more preferred embodiment, the gene is transcribed into an mRNA that encodes a CSP comprising an amino acid sequence of SEQ ID NO: 101 through 176. In another preferred embodiment, the allelic variant is a variant of a gene, wherein the gene is transcribed into an mRNA that is a CSNA. In a more preferred embodiment, the gene is transcribed into an mRNA that comprises the nucleic acid sequence of SEQ ID NO: 1 through 100. In a preferred embodiment, the allelic variant is a naturally-occurring allelic variant in the species of interest. In a more preferred embodiment, the species of interest is human.

By “nucleic acid molecule” it is also meant to be inclusive of a part of a nucleic acid sequence of the instant invention. The part may or may not encode a polypeptide, and may or may not encode a polypeptide that is a CSP. However, in a preferred embodiment, the part encodes a CSP. In one aspect, the invention comprises a part of a

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CSNA. In a second aspect, the invention comprises a part of a nucleic acid molecule that hybridizes or exhibits substantial sequence similarity to a CSNA. In a third aspect, the invention comprises a part of a nucleic acid molecule that is an allelic variant of a CSNA. In a fourth aspect, the invention comprises a part of a nucleic acid molecule that encodes  
5 a CSP. A part comprises at least 10 nucleotides, more preferably at least 15, 17, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400 or 500 nucleotides. The maximum size of a nucleic acid part is one nucleotide shorter than the sequence of the nucleic acid molecule encoding the full-length protein.

By "nucleic acid molecule" it is also meant to be inclusive of sequence that  
10 encoding a fusion protein, a homologous protein, a polypeptide fragment, a mutein or a polypeptide analog, as described below.

Nucleotide sequences of the instantly-described nucleic acids were determined by sequencing a DNA molecule that had resulted, directly or indirectly, from at least one enzymatic polymerization reaction (*e.g.*, reverse transcription and/or polymerase chain  
15 reaction) using an automated sequencer (such as the MegaBACE™ 1000, Molecular Dynamics, Sunnyvale, CA, USA). Further, all amino acid sequences of the polypeptides of the present invention were predicted by translation from the nucleic acid sequences so determined, unless otherwise specified.

In a preferred embodiment of the invention, the nucleic acid molecule contains  
20 modifications of the native nucleic acid molecule. These modifications include nonnative internucleoside bonds, post-synthetic modifications or altered nucleotide analogues. One having ordinary skill in the art would recognize that the type of modification that can be made will depend upon the intended use of the nucleic acid molecule. For instance, when the nucleic acid molecule is used as a hybridization probe,  
25 the range of such modifications will be limited to those that permit sequence-discriminating base pairing of the resulting nucleic acid. When used to direct expression of RNA or protein *in vitro* or *in vivo*, the range of such modifications will be limited to those that permit the nucleic acid to function properly as a polymerization substrate. When the isolated nucleic acid is used as a therapeutic agent, the modifications will be  
30 limited to those that do not confer toxicity upon the isolated nucleic acid.

In a preferred embodiment, isolated nucleic acid molecules can include nucleotide analogues that incorporate labels that are directly detectable, such as radiolabels or



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fluorophores, or nucleotide analogues that incorporate labels that can be visualized in a subsequent reaction, such as biotin or various haptens. In a more preferred embodiment, the labeled nucleic acid molecule may be used as a hybridization probe.

Common radiolabeled analogues include those labeled with  $^{33}\text{P}$ ,  $^{32}\text{P}$ , and  $^{35}\text{S}$ , such as  $^{-32}\text{P}$ -dATP,  $^{-32}\text{P}$ -dCTP,  $^{-32}\text{P}$ -dGTP,  $^{-32}\text{P}$ -dTTP,  $^{-32}\text{P}$ -3'dATP,  $^{-32}\text{P}$ -ATP,  $^{-32}\text{P}$ -CTP,  $^{-32}\text{P}$ -GTP,  $^{-32}\text{P}$ -UTP,  $^{-35}\text{S}$ -dATP,  $\alpha$ - $^{35}\text{S}$ -GTP,  $\alpha$ - $^{33}\text{P}$ -dATP, and the like.

Commercially available fluorescent nucleotide analogues readily incorporated into the nucleic acids of the present invention include Cy3-dCTP, Cy3-dUTP, Cy5-dCTP, Cy3-dUTP (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA), fluorescein-12-dUTP, tetramethylrhodamine-6-dUTP, Texas Red®-5-dUTP, Cascade Blue®-7-dUTP, BODIPY® FL-14-dUTP, BODIPY® TMR-14-dUTP, BODIPY® TR-14-dUTP, Rhodamine Green™-5-dUTP, Oregon Green® 488-5-dUTP, Texas Red®-12-dUTP, BODIPY® 630/650-14-dUTP, BODIPY® 650/665-14-dUTP, Alexa Fluor® 488-5-dUTP, Alexa Fluor® 532-5-dUTP, Alexa Fluor® 568-5-dUTP, Alexa Fluor® 594-5-dUTP, Alexa Fluor® 546-14-dUTP, fluorescein-12-UTP, tetramethylrhodamine-6-UTP, Texas Red®-5-UTP, Cascade Blue®-7-UTP, BODIPY® FL-14-UTP, BODIPY® TMR-14-UTP, BODIPY® TR-14-UTP, Rhodamine Green™-5-UTP, Alexa Fluor® 488-5-UTP, Alexa Fluor® 546-14-UTP (Molecular Probes, Inc. Eugene, OR, USA). One may also custom synthesize nucleotides having other fluorophores. See Henegariu *et al.*, *Nature Biotechnol.* 18: 345-348 (2000), the disclosure of which is incorporated herein by reference in its entirety.

Haptens that are commonly conjugated to nucleotides for subsequent labeling include biotin (biotin-11-dUTP, Molecular Probes, Inc., Eugene, OR, USA; biotin-21-UTP, biotin-21-dUTP, Clontech Laboratories, Inc., Palo Alto, CA, USA), digoxigenin (DIG-11-dUTP, alkali labile, DIG-11-UTP, Roche Diagnostics Corp., Indianapolis, IN, USA), and dinitrophenyl (dinitrophenyl-11-dUTP, Molecular Probes, Inc., Eugene, OR, USA).

Nucleic acid molecules can be labeled by incorporation of labeled nucleotide analogues into the nucleic acid. Such analogues can be incorporated by enzymatic polymerization, such as by nick translation, random priming, polymerase chain reaction (PCR), terminal transferase tailing, and end-filling of overhangs, for DNA molecules, and *in vitro* transcription driven, *e.g.*, from phage promoters, such as T7, T3, and SP6, for

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RNA molecules. Commercial kits are readily available for each such labeling approach. Analogues can also be incorporated during automated solid phase chemical synthesis. Labels can also be incorporated after nucleic acid synthesis, with the 5' phosphate and 3' hydroxyl providing convenient sites for post-synthetic covalent attachment of detectable  
5 labels.

Other post-synthetic approaches also permit internal labeling of nucleic acids. For example, fluorophores can be attached using a cisplatin reagent that reacts with the N7 of guanine residues (and, to a lesser extent, adenine bases) in DNA, RNA, and PNA to provide a stable coordination complex between the nucleic acid and fluorophore label  
10 (Universal Linkage System) (available from Molecular Probes, Inc., Eugene, OR, USA and Amersham Pharmacia Biotech, Piscataway, NJ, USA); *see Alers et al., Genes, Chromosomes & Cancer* 25: 301- 305 (1999); Jelsma *et al., J. NIH Res.* 5: 82 (1994); Van Belkum *et al., BioTechniques* 16: 148-153 (1994), incorporated herein by reference. As another example, nucleic acids can be labeled using a disulfide-containing linker  
15 (FastTag™ Reagent, Vector Laboratories, Inc., Burlingame, CA, USA) that is photo- or thermally-coupled to the target nucleic acid using aryl azide chemistry; after reduction, a free thiol is available for coupling to a hapten, fluorophore, sugar, affinity ligand, or other marker.

One or more independent or interacting labels can be incorporated into the  
20 nucleic acid molecules of the present invention. For example, both a fluorophore and a moiety that in proximity thereto acts to quench fluorescence can be included to report specific hybridization through release of fluorescence quenching or to report exonucleotidic excision. *See, e.g., Tyagi et al., Nature Biotechnol.* 14: 303-308 (1996); Tyagi *et al., Nature Biotechnol.* 16: 49-53 (1998); Sokol *et al., Proc. Natl. Acad. Sci.*  
25 *USA* 95: 11538-11543 (1998); Kostrikis *et al., Science* 279: 1228-1229 (1998); Marras *et al., Genet. Anal.* 14: 151-156 (1999); U. S. Patent 5,846,726; 5,925,517; 5,925,517; 5,723,591 and 5,538,848; Holland *et al., Proc. Natl. Acad. Sci. USA* 88: 7276-7280 (1991); Heid *et al., Genome Res.* 6(10): 986-94 (1996); Kuimelis *et al., Nucleic Acids Symp. Ser.* (37): 255-6 (1997); the disclosures of which are incorporated herein by  
30 reference in their entireties.

Nucleic acid molecules of the invention may be modified by altering one or more native phosphodiester internucleoside bonds to more nuclease-resistant, internucleoside

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bonds. See Hartmann *et al.* (eds.), Manual of Antisense Methodology: Perspectives in Antisense Science, Kluwer Law International (1999); Stein *et al.* (eds.), Applied Antisense Oligonucleotide Technology, Wiley-Liss (1998); Chadwick *et al.* (eds.), Oligonucleotides as Therapeutic Agents - Symposium No. 209, John Wiley & Son Ltd (1997); the disclosures of which are incorporated herein by reference in their entireties. Such altered internucleoside bonds are often desired for antisense techniques or for targeted gene correction. See Gamper *et al.*, *Nucl. Acids Res.* 28(21): 4332-4339 (2000), the disclosure of which is incorporated herein by reference in its entirety.

Modified oligonucleotide backbones include, without limitation,

10 phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having

15 normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U. S. Patents 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019;

20 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, the disclosures of which are incorporated herein by reference in their entireties. In a preferred embodiment, the modified internucleoside linkages may be used for antisense techniques.

25 Other modified oligonucleotide backbones do not include a phosphorus atom, but have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a

30 nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and

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methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH<sub>2</sub> component parts. Representative U.S. patents that teach the preparation of the above backbones include, but are not limited to, U.S. Patent 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437 and 5,677,439; the disclosures of which are incorporated herein by reference in their entireties.

In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage are replaced with novel groups, such as peptide nucleic acids (PNA). In PNA compounds, the phosphodiester backbone of the nucleic acid is replaced with an amide-containing backbone, in particular by repeating N-(2-aminoethyl) glycine units linked by amide bonds. Nucleobases are bound directly or indirectly to azanitrogen atoms of the amide portion of the backbone, typically by methylene carbonyl linkages. PNA can be synthesized using a modified peptide synthesis protocol. PNA oligomers can be synthesized by both Fmoc and tBoc methods. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patent 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Automated PNA synthesis is readily achievable on commercial synthesizers (*see, e.g.*, "PNA User's Guide," Rev. 2, February 1998, Perseptive Biosystems Part No. 60138, Applied Biosystems, Inc., Foster City, CA).

PNA molecules are advantageous for a number of reasons. First, because the PNA backbone is uncharged, PNA/DNA and PNA/RNA duplexes have a higher thermal stability than is found in DNA/DNA and DNA/RNA duplexes. The T<sub>m</sub> of a PNA/DNA or PNA/RNA duplex is generally 1°C higher per base pair than the T<sub>m</sub> of the corresponding DNA/DNA or DNA/RNA duplex (in 100 mM NaCl). Second, PNA molecules can also form stable PNA/DNA complexes at low ionic strength, under conditions in which DNA/DNA duplex formation does not occur. Third, PNA also demonstrates greater specificity in binding to complementary DNA because a PNA/DNA mismatch is more destabilizing than DNA/DNA mismatch. A single mismatch in mixed a PNA/DNA 15-mer lowers the T<sub>m</sub> by 8–20°C (15°C on average). In the corresponding DNA/DNA duplexes, a single mismatch lowers the T<sub>m</sub> by 4–16°C (11°C on average).

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Because PNA probes can be significantly shorter than DNA probes, their specificity is greater. Fourth, PNA oligomers are resistant to degradation by enzymes, and the lifetime of these compounds is extended both *in vivo* and *in vitro* because nucleases and proteases do not recognize the PNA polyamide backbone with nucleobase sidechains. *See, e.g.,*

- 5 Ray *et al.*, *FASEB J.* 14(9): 1041-60 (2000); Nielsen *et al.*, *Pharmacol Toxicol.* 86(1): 3-7 (2000); Larsen *et al.*, *Biochim Biophys Acta.* 1489(1): 159-66 (1999); Nielsen, *Curr. Opin. Struct. Biol.* 9(3): 353-7 (1999), and Nielsen, *Curr. Opin. Biotechnol.* 10(1): 71-5 (1999), the disclosures of which are incorporated herein by reference in their entireties.

- Nucleic acid molecules may be modified compared to their native structure  
10 throughout the length of the nucleic acid molecule or can be localized to discrete portions thereof. As an example of the latter, chimeric nucleic acids can be synthesized that have discrete DNA and RNA domains and that can be used for targeted gene repair and modified PCR reactions, as further described in U.S. Patents 5,760,012 and 5,731,181, Misra *et al.*, *Biochem.* 37: 1917-1925 (1998); and Finn *et al.*, *Nucl. Acids Res.* 24:  
15 3357-3363 (1996), the disclosures of which are incorporated herein by reference in their entireties.

- Unless otherwise specified, nucleic acids of the present invention can include any topological conformation appropriate to the desired use; the term thus explicitly comprehends, among others, single-stranded, double-stranded, triplexed, quadruplexed,  
20 partially double-stranded, partially-triplexed, partially-quadruplexed, branched, hairpinned, circular, and padlocked conformations. Padlock conformations and their utilities are further described in Banér *et al.*, *Curr. Opin. Biotechnol.* 12: 11-15 (2001); Escude *et al.*, *Proc. Natl. Acad. Sci. USA* 14: 96(19):10603-7 (1999); Nilsson *et al.*, *Science* 265(5181): 2085-8 (1994), the disclosures of which are incorporated herein by  
25 reference in their entireties. Triplex and quadruplex conformations, and their utilities, are reviewed in Praseuth *et al.*, *Biochim. Biophys. Acta.* 1489(1): 181-206 (1999); Fox, *Curr. Med. Chem.* 7(1): 17-37 (2000); Kochetkova *et al.*, *Methods Mol. Biol.* 130: 189-201 (2000); Chan *et al.*, *J. Mol. Med.* 75(4): 267-82 (1997), the disclosures of which are incorporated herein by reference in their entireties.

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*Methods for Using Nucleic Acid Molecules as Probes and Primers*

The isolated nucleic acid molecules of the present invention can be used as hybridization probes to detect, characterize, and quantify hybridizing nucleic acids in, and isolate hybridizing nucleic acids from, both genomic and transcript-derived nucleic acid samples. When free in solution, such probes are typically, but not invariably, detectably labeled; bound to a substrate, as in a microarray, such probes are typically, but not invariably unlabeled.

In one embodiment, the isolated nucleic acids of the present invention can be used as probes to detect and characterize gross alterations in the gene of a CSNA, such as deletions, insertions, translocations, and duplications of the CSNA genomic locus through fluorescence *in situ* hybridization (FISH) to chromosome spreads. See, e.g., Andreeff *et al.* (eds.), Introduction to Fluorescence In Situ Hybridization: Principles and Clinical Applications, John Wiley & Sons (1999), the disclosure of which is incorporated herein by reference in its entirety. The isolated nucleic acids of the present invention can be used as probes to assess smaller genomic alterations using, e.g., Southern blot detection of restriction fragment length polymorphisms. The isolated nucleic acid molecules of the present invention can be used as probes to isolate genomic clones that include the nucleic acid molecules of the present invention, which thereafter can be restriction mapped and sequenced to identify deletions, insertions, translocations, and substitutions (single nucleotide polymorphisms, SNPs) at the sequence level.

In another embodiment, the isolated nucleic acid molecules of the present invention can be used as probes to detect, characterize, and quantify CSNA in, and isolate CSNA from, transcript-derived nucleic acid samples. In one aspect, the isolated nucleic acid molecules of the present invention can be used as hybridization probes to detect, characterize by length, and quantify mRNA by Northern blot of total or poly-A<sup>+</sup>-selected RNA samples. In another aspect, the isolated nucleic acid molecules of the present invention can be used as hybridization probes to detect, characterize by location, and quantify mRNA by *in situ* hybridization to tissue sections. See, e.g., Schwarchzacher *et al.*, In Situ Hybridization, Springer-Verlag New York (2000), the disclosure of which is incorporated herein by reference in its entirety. In another preferred embodiment, the isolated nucleic acid molecules of the present invention can be used as hybridization probes to measure the representation of clones in a cDNA library or to isolate hybridizing

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nucleic acid molecules acids from cDNA libraries, permitting sequence level characterization of mRNAs that hybridize to CSNAs, including, without limitations, identification of deletions, insertions, substitutions, truncations, alternatively spliced forms and single nucleotide polymorphisms. In yet another preferred embodiment, the  
5 nucleic acid molecules of the instant invention may be used in microarrays.

All of the aforementioned probe techniques are well within the skill in the art, and are described at greater length in standard texts such as Sambrook (2001), *supra*; Ausubel (1999), *supra*; and Walker *et al.* (eds.), The Nucleic Acids Protocols Handbook, Humana Press (2000), the disclosures of which are incorporated herein by reference in  
10 their entirety.

Thus, in one embodiment, a nucleic acid molecule of the invention may be used as a probe or primer to identify or amplify a second nucleic acid molecule that selectively hybridizes to the nucleic acid molecule of the invention. In a preferred embodiment, the probe or primer is derived from a nucleic acid molecule encoding a CSP. In a more  
15 preferred embodiment, the probe or primer is derived from a nucleic acid molecule encoding a polypeptide having an amino acid sequence of SEQ ID NO: 101 through 176. In another preferred embodiment, the probe or primer is derived from a CSNA. In a more preferred embodiment, the probe or primer is derived from a nucleic acid molecule having a nucleotide sequence of SEQ ID NO: 1 through 100.

20 In general, a probe or primer is at least 10 nucleotides in length, more preferably at least 12, more preferably at least 14 and even more preferably at least 16 or 17 nucleotides in length. In an even more preferred embodiment, the probe or primer is at least 18 nucleotides in length, even more preferably at least 20 nucleotides and even more preferably at least 22 nucleotides in length. Primers and probes may also be longer  
25 in length. For instance, a probe or primer may be 25 nucleotides in length, or may be 30, 40 or 50 nucleotides in length. Methods of performing nucleic acid hybridization using oligonucleotide probes are well-known in the art. *See, e.g.*, Sambrook *et al.*, 1989, *supra*, Chapter 11 and pp. 11.31-11.32 and 11.40-11.44, which describes radiolabeling of short probes, and pp. 11.45-11.53, which describe hybridization conditions for oligonucleotide  
30 probes, including specific conditions for probe hybridization (pp. 11.50-11.51).

Methods of performing primer-directed amplification are also well-known in the art. Methods for performing the polymerase chain reaction (PCR) are compiled, *inter*

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- alia*, in McPherson, PCR Basics: From Background to Bench, Springer Verlag (2000); Innis *et al.* (eds.), PCR Applications: Protocols for Functional Genomics, Academic Press (1999); Gelfand *et al.* (eds.), PCR Strategies, Academic Press (1998); Newton *et al.*, PCR, Springer-Verlag New York (1997); Burke (ed.), PCR: Essential Techniques, John Wiley & Son Ltd (1996); White (ed.), PCR Cloning Protocols: From Molecular Cloning to Genetic Engineering, Vol. 67, Humana Press (1996); McPherson *et al.* (eds.), PCR 2: A Practical Approach, Oxford University Press, Inc. (1995); the disclosures of which are incorporated herein by reference in their entireties. Methods for performing RT-PCR are collected, *e.g.*, in Siebert *et al.* (eds.), Gene Cloning and Analysis by RT-PCR, Eaton Publishing Company/Bio Techniques Books Division, 1998; Siebert (ed.), PCR Technique: RT-PCR, Eaton Publishing Company/ BioTechniques Books (1995); the disclosure of which is incorporated herein by reference in its entirety.

PCR and hybridization methods may be used to identify and/or isolate allelic variants, homologous nucleic acid molecules and fragments of the nucleic acid molecules of the invention. PCR and hybridization methods may also be used to identify, amplify and/or isolate nucleic acid molecules that encode homologous proteins, analogs, fusion protein or muteins of the invention. The nucleic acid primers of the present invention can be used to prime amplification of nucleic acid molecules of the invention, using transcript-derived or genomic DNA as template.

The nucleic acid primers of the present invention can also be used, for example, to prime single base extension (SBE) for SNP detection (*See, e.g.*, U.S. Patent 6,004,744, the disclosure of which is incorporated herein by reference in its entirety).

Isothermal amplification approaches, such as rolling circle amplification, are also now well-described. *See, e.g.*, Schweitzer *et al.*, *Curr. Opin. Biotechnol.* 12(1): 21-7 (2001); U.S. Patents 5,854,033 and 5,714,320; and international patent publications WO 97/19193 and WO 00/15779, the disclosures of which are incorporated herein by reference in their entireties. Rolling circle amplification can be combined with other techniques to facilitate SNP detection. *See, e.g.*, Lizardi *et al.*, *Nature Genet.* 19(3): 225-32 (1998).

Nucleic acid molecules of the present invention may be bound to a substrate either covalently or noncovalently. The substrate can be porous or solid, planar or non-planar, unitary or distributed. The bound nucleic acid molecules may be used as



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hybridization probes, and may be labeled or unlabeled. In a preferred embodiment, the bound nucleic acid molecules are unlabeled.

In one embodiment, the nucleic acid molecule of the present invention is bound to a porous substrate, *e.g.*, a membrane, typically comprising nitrocellulose, nylon, or positively-charged derivatized nylon. The nucleic acid molecule of the present invention can be used to detect a hybridizing nucleic acid molecule that is present within a labeled nucleic acid sample, *e.g.*, a sample of transcript-derived nucleic acids. In another embodiment, the nucleic acid molecule is bound to a solid substrate, including, without limitation, glass, amorphous silicon, crystalline silicon or plastics. Examples of plastics include, without limitation, polymethylacrylic, polyethylene, polypropylene, polyacrylate, polymethylmethacrylate, polyvinylchloride, polytetrafluoroethylene, polystyrene, polycarbonate, polyacetal, polysulfone, celluloseacetate, cellulosenitrate, nitrocellulose, or mixtures thereof. The solid substrate may be any shape, including rectangular, disk-like and spherical. In a preferred embodiment, the solid substrate is a microscope slide or slide-shaped substrate.

The nucleic acid molecule of the present invention can be attached covalently to a surface of the support substrate or applied to a derivatized surface in a chaotropic agent that facilitates denaturation and adherence by presumed noncovalent interactions, or some combination thereof. The nucleic acid molecule of the present invention can be bound to a substrate to which a plurality of other nucleic acids are concurrently bound, hybridization to each of the plurality of bound nucleic acids being separately detectable. At low density, *e.g.* on a porous membrane, these substrate-bound collections are typically denominated macroarrays; at higher density, typically on a solid support, such as glass, these substrate bound collections of plural nucleic acids are colloquially termed microarrays. As used herein, the term microarray includes arrays of all densities. It is, therefore, another aspect of the invention to provide microarrays that include the nucleic acids of the present invention.

#### *Expression Vectors, Host Cells and Recombinant Methods of Producing Polypeptides*

Another aspect of the present invention relates to vectors that comprise one or more of the isolated nucleic acid molecules of the present invention, and host cells in which such vectors have been introduced.

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The vectors can be used, *inter alia*, for propagating the nucleic acids of the present invention in host cells (cloning vectors), for shuttling the nucleic acids of the present invention between host cells derived from disparate organisms (shuttle vectors), for inserting the nucleic acids of the present invention into host cell chromosomes  
5 (insertion vectors), for expressing sense or antisense RNA transcripts of the nucleic acids of the present invention *in vitro* or within a host cell, and for expressing polypeptides encoded by the nucleic acids of the present invention, alone or as fusions to heterologous polypeptides (expression vectors). Vectors of the present invention will often be suitable for several such uses.

10 Vectors are by now well-known in the art, and are described, *inter alia*, in Jones *et al.* (eds.), Vectors: Cloning Applications: Essential Techniques (Essential Techniques Series), John Wiley & Son Ltd. (1998); Jones *et al.* (eds.), Vectors: Expression Systems: Essential Techniques (Essential Techniques Series), John Wiley & Son Ltd. (1998); Gacesa *et al.*, Vectors: Essential Data, John Wiley & Sons Ltd. (1995); Cid-Arregui  
15 (eds.), Viral Vectors: Basic Science and Gene Therapy, Eaton Publishing Co. (2000); Sambrook (2001), *supra*; Ausubel (1999), *supra*; the disclosures of which are incorporated herein by reference in their entireties. Furthermore, an enormous variety of vectors are available commercially. Use of existing vectors and modifications thereof being well within the skill in the art, only basic features need be described here.

20 Nucleic acid sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate unicellular host. Expression control sequences are sequences which control the transcription, post-transcriptional events and translation of nucleic acid sequences. Such operative linking of a nucleic sequence of  
25 this invention to an expression control sequence, of course, includes, if not already part of the nucleic acid sequence, the provision of a translation initiation codon, ATG or GTG, in the correct reading frame upstream of the nucleic acid sequence.

A wide variety of host/expression vector combinations may be employed in expressing the nucleic acid sequences of this invention. Useful expression vectors, for  
30 example, may consist of segments of chromosomal, non-chromosomal and synthetic nucleic acid sequences.

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In one embodiment, prokaryotic cells may be used with an appropriate vector. Prokaryotic host cells are often used for cloning and expression. In a preferred embodiment, prokaryotic host cells include *E. coli*, *Pseudomonas*, *Bacillus* and *Streptomyces*. In a preferred embodiment, bacterial host cells are used to express the nucleic acid molecules of the instant invention. Useful expression vectors for bacterial hosts include bacterial plasmids, such as those from *E. coli*, *Bacillus* or *Streptomyces*, including pBluescript, pGEX-2T, pUC vectors, col E1, pCR1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as RP4, phage DNAs, *e.g.*, the numerous derivatives of phage lambda, *e.g.*, NM989,  $\lambda$ GT10 and  $\lambda$ GT11, and other phages, *e.g.*, M13 and filamentous single-stranded phage DNA. Where *E. coli* is used as host, selectable markers are, analogously, chosen for selectivity in gram negative bacteria: *e.g.*, typical markers confer resistance to antibiotics, such as ampicillin, tetracycline, chloramphenicol, kanamycin, streptomycin and zeocin; auxotrophic markers can also be used.

In other embodiments, eukaryotic host cells, such as yeast, insect, mammalian or plant cells, may be used. Yeast cells, typically *S. cerevisiae*, are useful for eukaryotic genetic studies, due to the ease of targeting genetic changes by homologous recombination and the ability to easily complement genetic defects using recombinantly expressed proteins. Yeast cells are useful for identifying interacting protein components, *e.g.* through use of a two-hybrid system. In a preferred embodiment, yeast cells are useful for protein expression. Vectors of the present invention for use in yeast will typically, but not invariably, contain an origin of replication suitable for use in yeast and a selectable marker that is functional in yeast. Yeast vectors include Yeast Integrating plasmids (*e.g.*, YIp5) and Yeast Replicating plasmids (the YRp and YEp series plasmids), Yeast Centromere plasmids (the YCp series plasmids), Yeast Artificial Chromosomes (YACs) which are based on yeast linear plasmids, denoted YLp, pGPD-2, 2 $\mu$  plasmids and derivatives thereof, and improved shuttle vectors such as those described in Gietz *et al.*, *Gene*, 74: 527-34 (1988) (YIplac, YEplac and YCplac). Selectable markers in yeast vectors include a variety of auxotrophic markers, the most common of which are (in *Saccharomyces cerevisiae*) URA3, HIS3, LEU2, TRP1 and LYS2, which complement specific auxotrophic mutations, such as *ura3-52*, *his3-D1*, *leu2-D1*, *trp1-D1* and *lys2-201*.

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Insect cells are often chosen for high efficiency protein expression. Where the host cells are from *Spodoptera frugiperda*, e.g., Sf9 and Sf21 cell lines, and expresSF™ cells (Protein Sciences Corp., Meriden, CT, USA)), the vector replicative strategy is typically based upon the baculovirus life cycle. Typically, baculovirus transfer vectors  
5 are used to replace the wild-type AcMNPV polyhedrin gene with a heterologous gene of interest. Sequences that flank the polyhedrin gene in the wild-type genome are positioned 5' and 3' of the expression cassette on the transfer vectors. Following co-transfection with AcMNPV DNA, a homologous recombination event occurs between these sequences resulting in a recombinant virus carrying the gene of interest and the  
10 polyhedrin or p10 promoter. Selection can be based upon visual screening for lacZ fusion activity.

In another embodiment, the host cells may be mammalian cells, which are particularly useful for expression of proteins intended as pharmaceutical agents, and for screening of potential agonists and antagonists of a protein or a physiological pathway.  
15 Mammalian vectors intended for autonomous extrachromosomal replication will typically include a viral origin, such as the SV40 origin (for replication in cell lines expressing the large T-antigen, such as COS1 and COS7 cells), the papillomavirus origin, or the EBV origin for long term episomal replication (for use, e.g., in 293-EBNA cells, which constitutively express the EBV EBNA-1 gene product and adenovirus E1A).  
20 Vectors intended for integration, and thus replication as part of the mammalian chromosome, can, but need not, include an origin of replication functional in mammalian cells, such as the SV40 origin. Vectors based upon viruses, such as adenovirus, adeno-associated virus, vaccinia virus, and various mammalian retroviruses, will typically replicate according to the viral replicative strategy. Selectable markers for use in  
25 mammalian cells include resistance to neomycin (G418), blasticidin, hygromycin and to zeocin, and selection based upon the purine salvage pathway using HAT medium.

Expression in mammalian cells can be achieved using a variety of plasmids, including pSV2, pBC12BI, and p91023, as well as lytic virus vectors (e.g., vaccinia virus, adeno virus, and baculovirus), episomal virus vectors (e.g., bovine papillomavirus),  
30 and retroviral vectors (e.g., murine retroviruses). Useful vectors for insect cells include baculoviral vectors and pVL 941.

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Plant cells can also be used for expression, with the vector replicon typically derived from a plant virus (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) and selectable markers chosen for suitability in plants.

It is known that codon usage of different host cells may be different. For example, a plant cell and a human cell may exhibit a difference in codon preference for encoding a particular amino acid. As a result, human mRNA may not be efficiently translated in a plant, bacteria or insect host cell. Therefore, another embodiment of this invention is directed to codon optimization. The codons of the nucleic acid molecules of the invention may be modified to resemble, as much as possible, genes naturally contained within the host cell without altering the amino acid sequence encoded by the nucleic acid molecule.

Any of a wide variety of expression control sequences may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include the expression control sequences associated with structural genes of the foregoing expression vectors. Expression control sequences that control transcription include, *e.g.*, promoters, enhancers and transcription termination sites. Expression control sequences in eukaryotic cells that control post-transcriptional events include splice donor and acceptor sites and sequences that modify the half-life of the transcribed RNA, *e.g.*, sequences that direct poly(A) addition or binding sites for RNA-binding proteins. Expression control sequences that control translation include ribosome binding sites, sequences which direct targeted expression of the polypeptide to or within particular cellular compartments, and sequences in the 5' and 3' untranslated regions that modify the rate or efficiency of translation.

Examples of useful expression control sequences for a prokaryote, *e.g.*, *E. coli*, will include a promoter, often a phage promoter, such as phage lambda pL promoter, the trc promoter, a hybrid derived from the trp and lac promoters, the bacteriophage T7 promoter (in *E. coli* cells engineered to express the T7 polymerase), the TAC or TRC system, the major operator and promoter regions of phage lambda, the control regions of fd coat protein, or the araBAD operon. Prokaryotic expression vectors may further include transcription terminators, such as the aspA terminator, and elements that facilitate translation, such as a consensus ribosome binding site and translation termination codon, Schomer *et al.*, *Proc. Natl. Acad. Sci. USA* 83: 8506-8510 (1986).

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Expression control sequences for yeast cells, typically *S. cerevisiae*, will include a yeast promoter, such as the CYC1 promoter, the GAL1 promoter, the GAL10 promoter, ADH1 promoter, the promoters of the yeast  $\mu$ -mating system, or the GPD promoter, and will typically have elements that facilitate transcription termination, such as the

5 transcription termination signals from the CYC1 or ADH1 gene.

Expression vectors useful for expressing proteins in mammalian cells will include a promoter active in mammalian cells. These promoters include those derived from mammalian viruses, such as the enhancer-promoter sequences from the immediate early gene of the human cytomegalovirus (CMV), the enhancer-promoter sequences from the

10 Rous sarcoma virus long terminal repeat (RSV LTR), the enhancer-promoter from SV40 or the early and late promoters of adenovirus. Other expression control sequences include the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase. Other expression control sequences include those from the gene comprising the CSNA of interest. Often, expression is enhanced by

15 incorporation of polyadenylation sites, such as the late SV40 polyadenylation site and the polyadenylation signal and transcription termination sequences from the bovine growth hormone (BGH) gene, and ribosome binding sites. Furthermore, vectors can include introns, such as intron II of rabbit  $\beta$ -globin gene and the SV40 splice elements.

Preferred nucleic acid vectors also include a selectable or amplifiable marker

20 gene and means for amplifying the copy number of the gene of interest. Such marker genes are well-known in the art. Nucleic acid vectors may also comprise stabilizing sequences (*e.g.*, ori- or ARS-like sequences and telomere-like sequences), or may alternatively be designed to favor directed or non-directed integration into the host cell genome. In a preferred embodiment, nucleic acid sequences of this invention are inserted

25 in frame into an expression vector that allows high level expression of an RNA which encodes a protein comprising the encoded nucleic acid sequence of interest. Nucleic acid cloning and sequencing methods are well-known to those of skill in the art and are described in an assortment of laboratory manuals, including Sambrook (1989), *supra*, Sambrook (2000), *supra*; and Ausubel (1992), *supra*, Ausubel (1999), *supra*. Product

30 information from manufacturers of biological, chemical and immunological reagents also provide useful information.

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Expression vectors may be either constitutive or inducible. Inducible vectors include either naturally inducible promoters, such as the *trc* promoter, which is regulated by the *lac* operon, and the *pL* promoter, which is regulated by tryptophan, the MMTV-LTR promoter, which is inducible by dexamethasone, or can contain synthetic  
5 promoters and/or additional elements that confer inducible control on adjacent promoters. Examples of inducible synthetic promoters are the hybrid *Plac/ara-1* promoter and the *PLtetO-1* promoter. The *PLtetO-1* promoter takes advantage of the high expression levels from the *PL* promoter of phage lambda, but replaces the lambda repressor sites with two copies of operator 2 of the *Tn10* tetracycline resistance operon, causing this promoter to  
10 be tightly repressed by the Tet repressor protein and induced in response to tetracycline (Tc) and Tc derivatives such as anhydrotetracycline. Vectors may also be inducible because they contain hormone response elements, such as the glucocorticoid response element (GRE) and the estrogen response element (ERE), which can confer hormone inducibility where vectors are used for expression in cells having the respective hormone  
15 receptors. To reduce background levels of expression, elements responsive to ecdysone, an insect hormone, can be used instead, with coexpression of the ecdysone receptor.

In one aspect of the invention, expression vectors can be designed to fuse the expressed polypeptide to small protein tags that facilitate purification and/or visualization. Tags that facilitate purification include a polyhistidine tag that facilitates  
20 purification of the fusion protein by immobilized metal affinity chromatography, for example using NiNTA resin (Qiagen Inc., Valencia, CA, USA) or TALON™ resin (cobalt immobilized affinity chromatography medium, Clontech Labs, Palo Alto, CA, USA). The fusion protein can include a chitin-binding tag and self-excising intein, permitting chitin-based purification with self-removal of the fused tag (IMPACT™  
25 system, New England Biolabs, Inc., Beverly, MA, USA). Alternatively, the fusion protein can include a calmodulin-binding peptide tag, permitting purification by calmodulin affinity resin (Stratagene, La Jolla, CA, USA), or a specifically excisable fragment of the biotin carboxylase carrier protein, permitting purification of *in vivo* biotinylated protein using an avidin resin and subsequent tag removal (Promega,  
30 Madison, WI, USA). As another useful alternative, the proteins of the present invention can be expressed as a fusion protein with glutathione-S-transferase, the affinity and specificity of binding to glutathione permitting purification using glutathione affinity

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resins, such as Glutathione-Superflow Resin (Clontech Laboratories, Palo Alto, CA, USA), with subsequent elution with free glutathione. Other tags include, for example, the Xpress epitope, detectable by anti-Xpress antibody (Invitrogen, Carlsbad, CA, USA), a myc tag, detectable by anti-myc tag antibody, the V5 epitope, detectable by anti-V5  
5 antibody (Invitrogen, Carlsbad, CA, USA), FLAG® epitope, detectable by anti-FLAG® antibody (Stratagene, La Jolla, CA, USA), and the HA epitope.

For secretion of expressed proteins, vectors can include appropriate sequences that encode secretion signals, such as leader peptides. For example, the pSecTag2  
10 vectors (Invitrogen, Carlsbad, CA, USA) are 5.2 kb mammalian expression vectors that carry the secretion signal from the V-J2-C region of the mouse Ig kappa-chain for efficient secretion of recombinant proteins from a variety of mammalian cell lines.

Expression vectors can also be designed to fuse proteins encoded by the heterologous nucleic acid insert to polypeptides that are larger than purification and/or  
15 identification tags. Useful fusion proteins include those that permit display of the encoded protein on the surface of a phage or cell, fusion to intrinsically fluorescent proteins, such as those that have a green fluorescent protein (GFP)-like chromophore, fusions to the IgG Fc region, and fusion proteins for use in two hybrid systems.

Vectors for phage display fuse the encoded polypeptide to, *e.g.*, the gene III protein (pIII) or gene VIII protein (pVIII) for display on the surface of filamentous  
20 phage, such as M13. *See* Barbas *et al.*, Phage Display: A Laboratory Manual, Cold Spring Harbor Laboratory Press (2001); Kay *et al.* (eds.), Phage Display of Peptides and Proteins: A Laboratory Manual, Academic Press, Inc., (1996); Abelson *et al.* (eds.), Combinatorial Chemistry (Methods in Enzymology, Vol. 267) Academic Press (1996).  
Vectors for yeast display, *e.g.* the pYD1 yeast display vector (Invitrogen, Carlsbad, CA,  
25 USA), use the  $\alpha$ -agglutinin yeast adhesion receptor to display recombinant protein on the surface of *S. cerevisiae*. Vectors for mammalian display, *e.g.*, the pDisplay™ vector (Invitrogen, Carlsbad, CA, USA), target recombinant proteins using an N-terminal cell surface targeting signal and a C-terminal transmembrane anchoring domain of platelet derived growth factor receptor.

30 A wide variety of vectors now exist that fuse proteins encoded by heterologous nucleic acids to the chromophore of the substrate-independent, intrinsically fluorescent green fluorescent protein from *Aequorea victoria* ("GFP") and its variants. The GFP-like



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chromophore can be selected from GFP-like chromophores found in naturally occurring proteins, such as *A. victoria* GFP (GenBank accession number AAA27721), *Renilla reniformis* GFP, FP583 (GenBank accession no. AF168419) (DsRed), FP593 (AF272711), FP483 (AF168420), FP484 (AF168424), FP595 (AF246709), FP486 (AF168421), FP538 (AF168423), and FP506 (AF168422), and need include only so much of the native protein as is needed to retain the chromophore's intrinsic fluorescence. Methods for determining the minimal domain required for fluorescence are known in the art. *See Li et al., J. Biol. Chem.* 272: 28545-28549 (1997). Alternatively, the GFP-like chromophore can be selected from GFP-like chromophores modified from those found in nature. The methods for engineering such modified GFP-like chromophores and testing them for fluorescence activity, both alone and as part of protein fusions, are well-known in the art. *See Heim et al., Curr. Biol.* 6: 178-182 (1996) and Palm *et al., Methods Enzymol.* 302: 378-394 (1999), incorporated herein by reference in its entirety. A variety of such modified chromophores are now commercially available and can readily be used in the fusion proteins of the present invention. These include EGFP ("enhanced GFP"), EBFP ("enhanced blue fluorescent protein"), BFP2, EYFP ("enhanced yellow fluorescent protein"), ECFP ("enhanced cyan fluorescent protein") or Citrine. EGFP (*see, e.g., Cormack et al., Gene* 173: 33-38 (1996); United States Patent Nos. 6,090,919 and 5,804,387) is found on a variety of vectors, both plasmid and viral, which are available commercially (Clontech Labs, Palo Alto, CA, USA); EBFP is optimized for expression in mammalian cells whereas BFP2, which retains the original jellyfish codons, can be expressed in bacteria (*see, e.g., Heim et al., Curr. Biol.* 6: 178-182 (1996) and Cormack *et al., Gene* 173: 33-38 (1996)). Vectors containing these blue-shifted variants are available from Clontech Labs (Palo Alto, CA, USA). Vectors containing EYFP, ECFP (*see, e.g., Heim et al., Curr. Biol.* 6: 178-182 (1996); Miyawaki *et al., Nature* 388: 882-887 (1997)) and Citrine (*see, e.g., Heikal et al., Proc. Natl. Acad. Sci. USA* 97: 11996-12001 (2000)) are also available from Clontech Labs. The GFP-like chromophore can also be drawn from other modified GFPs, including those described in U.S. Patents 6,124,128; 6,096,865; 6,090,919; 6,066,476; 6,054,321; 6,027,881; 5,968,750; 5,874,304; 5,804,387; 5,777,079; 5,741,668; and 5,625,048, the disclosures of which are incorporated herein by reference in their entireties. *See also Conn (ed.), Green Fluorescent Protein* (Methods in

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Enzymology, Vol. 302), Academic Press, Inc. (1999). The GFP-like chromophore of each of these GFP variants can usefully be included in the fusion proteins of the present invention.

5 Fusions to the IgG Fc region increase serum half life of protein pharmaceutical products through interaction with the FcRn receptor (also denominated the FcRp receptor and the Brambell receptor, FcRb), further described in International Patent Application Nos. WO 97/43316, WO 97/34631, WO 96/32478, WO 96/18412.

10 For long-term, high-yield recombinant production of the proteins, protein fusions, and protein fragments of the present invention, stable expression is preferred. Stable expression is readily achieved by integration into the host cell genome of vectors having selectable markers, followed by selection of these integrants. Vectors such as pUB6/V5-His A, B, and C (Invitrogen, Carlsbad, CA, USA) are designed for high-level stable expression of heterologous proteins in a wide range of mammalian tissue types and cell lines. pUB6/V5-His uses the promoter/enhancer sequence from the human ubiquitin  
15 C gene to drive expression of recombinant proteins: expression levels in 293, CHO, and NIH3T3 cells are comparable to levels from the CMV and human EF-1a promoters. The bsd gene permits rapid selection of stably transfected mammalian cells with the potent antibiotic blasticidin.

20 Replication incompetent retroviral vectors, typically derived from Moloney murine leukemia virus, also are useful for creating stable transfectants having integrated provirus. The highly efficient transduction machinery of retroviruses, coupled with the availability of a variety of packaging cell lines such as RetroPack™ PT 67, EcoPack2™-293, AmphoPack-293, and GP2-293 cell lines (all available from Clontech Laboratories, Palo Alto, CA, USA), allow a wide host range to be infected with high efficiency;  
25 varying the multiplicity of infection readily adjusts the copy number of the integrated provirus.

Of course, not all vectors and expression control sequences will function equally well to express the nucleic acid sequences of this invention. Neither will all hosts function equally well with the same expression system. However, one of skill in the art  
30 may make a selection among these vectors, expression control sequences and hosts without undue experimentation and without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must

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be replicated in it. The vector's copy number, the ability to control that copy number, the ability to control integration, if any, and the expression of any other proteins encoded by the vector, such as antibiotic or other selection markers, should also be considered. The present invention further includes host cells comprising the vectors of the present  
5 invention, either present episomally within the cell or integrated, in whole or in part, into the host cell chromosome. Among other considerations, some of which are described above, a host cell strain may be chosen for its ability to process the expressed protein in the desired fashion. Such post-translational modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation,  
10 and acylation, and it is an aspect of the present invention to provide CSPs with such post-translational modifications.

Polypeptides of the invention may be post-translationally modified. Post-translational modifications include phosphorylation of amino acid residues serine, threonine and/or tyrosine, N-linked and/or O-linked glycosylation, methylation,  
15 acetylation, prenylation, methylation, acetylation, arginylation, ubiquination and racemization. One may determine whether a polypeptide of the invention is likely to be post-translationally modified by analyzing the sequence of the polypeptide to determine if there are peptide motifs indicative of sites for post-translational modification. There are a number of computer programs that permit prediction of post-translational  
20 modifications. See, e.g., [www.expasy.org](http://www.expasy.org) (accessed August 31, 2001), which includes PSORT, for prediction of protein sorting signals and localization sites, SignalP, for prediction of signal peptide cleavage sites, MITOPROT and Predotar, for prediction of mitochondrial targeting sequences, NetOGlyc, for prediction of type O-glycosylation sites in mammalian proteins, big-PI Predictor and DGPI, for prediction of prenylation-  
25 anchor and cleavage sites, and NetPhos, for prediction of Ser, Thr and Tyr phosphorylation sites in eukaryotic proteins. Other computer programs, such as those included in GCG, also may be used to determine post-translational modification peptide motifs.

General examples of types of post-translational modifications may be found in  
30 web sites such as the Delta Mass database <http://www.abrf.org/ABRF/Research/Committees/deltamass/deltamass.html> (accessed October 19, 2001); "GlycoSuiteDB: a new curated relational database of glycoprotein glycan structures and their biological

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sources" Cooper et al. *Nucleic Acids Res.* 29; 332-335 (2001) and <http://www.glycosuite.com/> (accessed October 19, 2001); "O-GLYCBASE version 4.0: a revised database of O-glycosylated proteins" Gupta et al. *Nucleic Acids Research*, 27: 370-372 (1999) and <http://www.cbs.dtu.dk/databases/OGLYCBASE/> (accessed October 19, 2001); "PhosphoBase, a database of phosphorylation sites: release 2.0.", Kreegipuu et al. *Nucleic Acids Res* 27(1):237-239 (1999) and <http://www.cbs.dtu.dk/databases/PhosphoBase/> (accessed October 19, 2001); or <http://pir.georgetown.edu/pirwww/search/textresid.html> (accessed October 19, 2001).

Tumorigenesis is often accompanied by alterations in the post-translational modifications of proteins. Thus, in another embodiment, the invention provides polypeptides from cancerous cells or tissues that have altered post-translational modifications compared to the post-translational modifications of polypeptides from normal cells or tissues. A number of altered post-translational modifications are known. One common alteration is a change in phosphorylation state, wherein the polypeptide from the cancerous cell or tissue is hyperphosphorylated or hypophosphorylated compared to the polypeptide from a normal tissue, or wherein the polypeptide is phosphorylated on different residues than the polypeptide from a normal cell. Another common alteration is a change in glycosylation state, wherein the polypeptide from the cancerous cell or tissue has more or less glycosylation than the polypeptide from a normal tissue, and/or wherein the polypeptide from the cancerous cell or tissue has a different type of glycosylation than the polypeptide from a noncancerous cell or tissue. Changes in glycosylation may be critical because carbohydrate-protein and carbohydrate-carbohydrate interactions are important in cancer cell progression, dissemination and invasion. See, e.g., Barchi, *Curr. Pharm. Des.* 6: 485-501 (2000), Verma, *Cancer Biochem. Biophys.* 14: 151-162 (1994) and Dennis et al., *Bioessays* 5: 412-421 (1999).

Another post-translational modification that may be altered in cancer cells is prenylation. Prenylation is the covalent attachment of a hydrophobic prenyl group (either farnesyl or geranylgeranyl) to a polypeptide. Prenylation is required for localizing a protein to a cell membrane and is often required for polypeptide function. For instance, the Ras superfamily of GTPase signaling proteins must be prenylated for function in a cell. See, e.g., Prendergast et al., *Semin. Cancer Biol.* 10: 443-452 (2000) and Khwaja et al., *Lancet* 355: 741-744 (2000).

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Other post-translation modifications that may be altered in cancer cells include, without limitation, polypeptide methylation, acetylation, arginylation or racemization of amino acid residues. In these cases, the polypeptide from the cancerous cell may exhibit either increased or decreased amounts of the post-translational modification compared to the corresponding polypeptides from noncancerous cells.

Other polypeptide alterations in cancer cells include abnormal polypeptide cleavage of proteins and aberrant protein-protein interactions. Abnormal polypeptide cleavage may be cleavage of a polypeptide in a cancerous cell that does not usually occur in a normal cell, or a lack of cleavage in a cancerous cell, wherein the polypeptide is cleaved in a normal cell. Aberrant protein-protein interactions may be either covalent cross-linking or non-covalent binding between proteins that do not normally bind to each other. Alternatively, in a cancerous cell, a protein may fail to bind to another protein to which it is bound in a noncancerous cell. Alterations in cleavage or in protein-protein interactions may be due to over- or underproduction of a polypeptide in a cancerous cell compared to that in a normal cell, or may be due to alterations in post-translational modifications (see above) of one or more proteins in the cancerous cell. See, e.g., Henschen-Edman, *Ann. N.Y. Acad. Sci.* 936: 580-593 (2001).

Alterations in polypeptide post-translational modifications, as well as changes in polypeptide cleavage and protein-protein interactions, may be determined by any method known in the art. For instance, alterations in phosphorylation may be determined by using anti-phosphoserine, anti-phosphothreonine or anti-phosphotyrosine antibodies or by amino acid analysis. Glycosylation alterations may be determined using antibodies specific for different sugar residues, by carbohydrate sequencing, or by alterations in the size of the glycoprotein, which can be determined by, e.g., SDS polyacrylamide gel electrophoresis (PAGE). Other alterations of post-translational modifications, such as prenylation, racemization, methylation, acetylation and arginylation, may be determined by chemical analysis, protein sequencing, amino acid analysis, or by using antibodies specific for the particular post-translational modifications. Changes in protein-protein interactions and in polypeptide cleavage may be analyzed by any method known in the art including, without limitation, non-denaturing PAGE (for non-covalent protein-protein interactions), SDS PAGE (for covalent protein-protein interactions and protein cleavage), chemical cleavage, protein sequencing or immunoassays.

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In another embodiment, the invention provides polypeptides that have been post-translationally modified. In one embodiment, polypeptides may be modified enzymatically or chemically, by addition or removal of a post-translational modification. For example, a polypeptide may be glycosylated or deglycosylated enzymatically.

5 Similarly, polypeptides may be phosphorylated using a purified kinase, such as a MAP kinase (e.g. p38, ERK, or JNK) or a tyrosine kinase (e.g., Src or erbB2). A polypeptide may also be modified through synthetic chemistry. Alternatively, one may isolate the polypeptide of interest from a cell or tissue that expresses the polypeptide with the desired post-translational modification. In another embodiment, a nucleic acid molecule  
10 encoding the polypeptide of interest is introduced into a host cell that is capable of post-translationally modifying the encoded polypeptide in the desired fashion. If the polypeptide does not contain a motif for a desired post-translational modification, one may alter the post-translational modification by mutating the nucleic acid sequence of a nucleic acid molecule encoding the polypeptide so that it contains a site for the desired  
15 post-translational modification. Amino acid sequences that may be post-translationally modified are known in the art. See, e.g., the programs described above on the website [www.expasy.org](http://www.expasy.org). The nucleic acid molecule is then be introduced into a host cell that is capable of post-translationally modifying the encoded polypeptide. Similarly, one may delete sites that are post-translationally modified by either mutating the nucleic acid  
20 sequence so that the encoded polypeptide does not contain the post-translational modification motif, or by introducing the native nucleic acid molecule into a host cell that is not capable of post-translationally modifying the encoded polypeptide.

In selecting an expression control sequence, a variety of factors should also be considered. These include, for example, the relative strength of the sequence, its  
25 controllability, and its compatibility with the nucleic acid sequence of this invention, particularly with regard to potential secondary structures. Unicellular hosts should be selected by consideration of their compatibility with the chosen vector, the toxicity of the product coded for by the nucleic acid sequences of this invention, their secretion characteristics, their ability to fold the polypeptide correctly, their fermentation or culture  
30 requirements, and the ease of purification from them of the products coded for by the nucleic acid sequences of this invention.

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The recombinant nucleic acid molecules and more particularly, the expression vectors of this invention may be used to express the polypeptides of this invention as recombinant polypeptides in a heterologous host cell. The polypeptides of this invention may be full-length or less than full-length polypeptide fragments recombinantly  
5 expressed from the nucleic acid sequences according to this invention. Such polypeptides include analogs, derivatives and muteins that may or may not have biological activity.

Vectors of the present invention will also often include elements that permit *in vitro* transcription of RNA from the inserted heterologous nucleic acid. Such vectors  
10 typically include a phage promoter, such as that from T7, T3, or SP6, flanking the nucleic acid insert. Often two different such promoters flank the inserted nucleic acid, permitting separate *in vitro* production of both sense and antisense strands.

Transformation and other methods of introducing nucleic acids into a host cell (*e.g.*, conjugation, protoplast transformation or fusion, transfection, electroporation,  
15 liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion) can be accomplished by a variety of methods which are well-known in the art (*See*, for instance, Ausubel, *supra*, and Sambrook *et al.*, *supra*). Bacterial, yeast, plant or mammalian cells are transformed or transfected with an expression vector, such as a plasmid, a cosmid, or the like, wherein the expression vector  
20 comprises the nucleic acid of interest. Alternatively, the cells may be infected by a viral expression vector comprising the nucleic acid of interest. Depending upon the host cell, vector, and method of transformation used, transient or stable expression of the polypeptide will be constitutive or inducible. One having ordinary skill in the art will be able to decide whether to express a polypeptide transiently or stably, and whether to  
25 express the protein constitutively or inducibly.

A wide variety of unicellular host cells are useful in expressing the DNA sequences of this invention. These hosts may include well-known eukaryotic and prokaryotic hosts, such as strains of, fungi, yeast, insect cells such as *Spodoptera frugiperda* (SF9), animal cells such as CHO, as well as plant cells in tissue culture.  
30 Representative examples of appropriate host cells include, but are not limited to, bacterial cells, such as *E. coli*, *Caulobacter crescentus*, *Streptomyces* species, and *Salmonella typhimurium*; yeast cells, such as *Saccharomyces cerevisiae*, *Schizosaccharomyces*

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*pombe*, *Pichia pastoris*, *Pichia methanolica*; insect cell lines, such as those from *Spodoptera frugiperda*, e.g., Sf9 and Sf21 cell lines, and expresSF™ cells (Protein Sciences Corp., Meriden, CT, USA), *Drosophila* S2 cells, and *Trichoplusia ni* High Five® Cells (Invitrogen, Carlsbad, CA, USA); and mammalian cells. Typical

5 mammalian cells include BHK cells, BSC 1 cells, BSC 40 cells, BMT 10 cells, VERO cells, COS1 cells, COS7 cells, Chinese hamster ovary (CHO) cells, 3T3 cells, NIH 3T3 cells, 293 cells, HEPG2 cells, HeLa cells, L cells, MDCK cells, HEK293 cells, WI38 cells, murine ES cell lines (e.g., from strains 129/SV, C57/BL6, DBA-1, 129/SVJ), K562 cells, Jurkat cells, and BW5147 cells. Other mammalian cell lines are well-known and

10 readily available from the American Type Culture Collection (ATCC) (Manassas, VA, USA) and the National Institute of General Medical Sciences (NIGMS) Human Genetic Cell Repository at the Coriell Cell Repositories (Camden, NJ, USA). Cells or cell lines derived from colon are particularly preferred because they may provide a more native post-translational processing. Particularly preferred are human colon cells.

15 Particular details of the transfection, expression and purification of recombinant proteins are well documented and are understood by those of skill in the art. Further details on the various technical aspects of each of the steps used in recombinant production of foreign genes in bacterial cell expression systems can be found in a number of texts and laboratory manuals in the art. See, e.g., Ausubel (1992), *supra*, Ausubel

20 (1999), *supra*, Sambrook (1989), *supra*, and Sambrook (2001), *supra*, herein incorporated by reference.

Methods for introducing the vectors and nucleic acids of the present invention into the host cells are well-known in the art; the choice of technique will depend primarily upon the specific vector to be introduced and the host cell chosen.

25 Nucleic acid molecules and vectors may be introduced into prokaryotes, such as *E. coli*, in a number of ways. For instance, phage lambda vectors will typically be packaged using a packaging extract (e.g., Gigapack® packaging extract, Stratagene, La Jolla, CA, USA), and the packaged virus used to infect *E. coli*.

Plasmid vectors will typically be introduced into chemically competent or

30 electrocompetent bacterial cells. *E. coli* cells can be rendered chemically competent by treatment, e.g., with CaCl<sub>2</sub>, or a solution of Mg<sup>2+</sup>, Mn<sup>2+</sup>, Ca<sup>2+</sup>, Rb<sup>+</sup> or K<sup>+</sup>, dimethyl sulfoxide, dithiothreitol, and hexamine cobalt (III), Hanahan, *J. Mol. Biol.* 166(4):557-80



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(1983), and vectors introduced by heat shock. A wide variety of chemically competent strains are also available commercially (*e.g.*, Epicurian Coli® XL10-Gold® Ultracompetent Cells (Stratagene, La Jolla, CA, USA); DH5 competent cells (Clontech Laboratories, Palo Alto, CA, USA); and TOP10 Chemically Competent *E. coli* Kit (Invitrogen, Carlsbad, CA, USA)). Bacterial cells can be rendered electrocompetent, that is, competent to take up exogenous DNA by electroporation, by various pre-pulse treatments; vectors are introduced by electroporation followed by subsequent outgrowth in selected media. An extensive series of protocols is provided online in Electroprotocols (BioRad, Richmond, CA, USA) ([http://www.biorad.com/LifeScience/pdf/](http://www.biorad.com/LifeScience/pdf/New_Gene_Pulser.pdf)  
New\_Gene\_Pulser.pdf).

Vectors can be introduced into yeast cells by spheroplasting, treatment with lithium salts, electroporation, or protoplast fusion. Spheroplasts are prepared by the action of hydrolytic enzymes such as snail-gut extract, usually denoted Glusulase, or Zymolyase, an enzyme from *Arthrobacter luteus*, to remove portions of the cell wall in the presence of osmotic stabilizers, typically 1 M sorbitol. DNA is added to the spheroplasts, and the mixture is co-precipitated with a solution of polyethylene glycol (PEG) and  $\text{Ca}^{2+}$ . Subsequently, the cells are resuspended in a solution of sorbitol, mixed with molten agar and then layered on the surface of a selective plate containing sorbitol.

For lithium-mediated transformation, yeast cells are treated with lithium acetate, which apparently permeabilizes the cell wall, DNA is added and the cells are co-precipitated with PEG. The cells are exposed to a brief heat shock, washed free of PEG and lithium acetate, and subsequently spread on plates containing ordinary selective medium. Increased frequencies of transformation are obtained by using specially-prepared single-stranded carrier DNA and certain organic solvents. Schiestl *et al.*, *Curr. Genet.* 16(5-6): 339-46 (1989).

For electroporation, freshly-grown yeast cultures are typically washed, suspended in an osmotic protectant, such as sorbitol, mixed with DNA, and the cell suspension pulsed in an electroporation device. Subsequently, the cells are spread on the surface of plates containing selective media. Becker *et al.*, *Methods Enzymol.* 194: 182-187 (1991). The efficiency of transformation by electroporation can be increased over 100-fold by using PEG, single-stranded carrier DNA and cells that are in late log-phase of growth. Larger constructs, such as YACs, can be introduced by protoplast fusion.

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Mammalian and insect cells can be directly infected by packaged viral vectors, or transfected by chemical or electrical means. For chemical transfection, DNA can be coprecipitated with  $\text{CaPO}_4$  or introduced using liposomal and nonliposomal lipid-based agents. Commercial kits are available for  $\text{CaPO}_4$  transfection (CalPhos™ Mammalian Transfection Kit, Clontech Laboratories, Palo Alto, CA, USA), and lipid-mediated transfection can be practiced using commercial reagents, such as LIPOFECTAMINE™ 2000, LIPOFECTAMINE™ Reagent, CELLFECTIN® Reagent, and LIPOFECTIN® Reagent (Invitrogen, Carlsbad, CA, USA), DOTAP Liposomal Transfection Reagent, FuGENE 6, X-tremeGENE Q2, DOSPER, (Roche Molecular Biochemicals, Indianapolis, IN USA), Effectene™, PolyFect®, Superfect® (Qiagen, Inc., Valencia, CA, USA). Protocols for electroporating mammalian cells can be found online in Electroprotocols (Bio-Rad, Richmond, CA, USA) ([http://www.bio-rad.com/LifeScience/pdf/New\\_Gene\\_Pulser.pdf](http://www.bio-rad.com/LifeScience/pdf/New_Gene_Pulser.pdf)); Norton *et al.* (eds.), Gene Transfer Methods: Introducing DNA into Living Cells and Organisms, BioTechniques Books, Eaton Publishing Co. (2000); incorporated herein by reference in its entirety. Other transfection techniques include transfection by particle bombardment and microinjection. *See, e.g.,* Cheng *et al.*, *Proc. Natl. Acad. Sci. USA* 90(10): 4455-9 (1993); Yang *et al.*, *Proc. Natl. Acad. Sci. USA* 87(24): 9568-72 (1990).

Production of the recombinantly produced proteins of the present invention can optionally be followed by purification.

Purification of recombinantly expressed proteins is now well by those skilled in the art. *See, e.g.,* Thorner *et al.* (eds.), Applications of Chimeric Genes and Hybrid Proteins, Part A: Gene Expression and Protein Purification (Methods in Enzymology, Vol. 326), Academic Press (2000); Harbin (ed.), Cloning, Gene Expression and Protein Purification : Experimental Procedures and Process Rationale, Oxford Univ. Press (2001); Marshak *et al.*, Strategies for Protein Purification and Characterization: A Laboratory Course Manual, Cold Spring Harbor Laboratory Press (1996); and Roe (ed.), Protein Purification Applications, Oxford University Press (2001); the disclosures of which are incorporated herein by reference in their entireties, and thus need not be detailed here.

Briefly, however, if purification tags have been fused through use of an expression vector that appends such tags, purification can be effected, at least in part, by

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means appropriate to the tag, such as use of immobilized metal affinity chromatography for polyhistidine tags. Other techniques common in the art include ammonium sulfate fractionation, immunoprecipitation, fast protein liquid chromatography (FPLC), high performance liquid chromatography (HPLC), and preparative gel electrophoresis.

## 5 Polypeptides

Another object of the invention is to provide polypeptides encoded by the nucleic acid molecules of the instant invention. In a preferred embodiment, the polypeptide is a colon specific polypeptide (CSP). In an even more preferred embodiment, the polypeptide is derived from a polypeptide comprising the amino acid sequence of SEQ  
10 ID NO: 101 through 176. A polypeptide as defined herein may be produced recombinantly, as discussed *supra*, may be isolated from a cell that naturally expresses the protein, or may be chemically synthesized following the teachings of the specification and using methods well-known to those having ordinary skill in the art.

In another aspect, the polypeptide may comprise a fragment of a polypeptide,  
15 wherein the fragment is as defined herein. In a preferred embodiment, the polypeptide fragment is a fragment of a CSP. In a more preferred embodiment, the fragment is derived from a polypeptide comprising the amino acid sequence of SEQ ID NO: 101 through 176. A polypeptide that comprises only a fragment of an entire CSP may or may not be a polypeptide that is also a CSP. For instance, a full-length polypeptide may be  
20 colon-specific, while a fragment thereof may be found in other tissues as well as in colon. A polypeptide that is not a CSP, whether it is a fragment, analog, mutein, homologous protein or derivative, is nevertheless useful, especially for immunizing animals to prepare anti-CSP antibodies. However, in a preferred embodiment, the part or fragment is a CSP. Methods of determining whether a polypeptide is a CSP are described *infra*.

25 Fragments of at least 6 contiguous amino acids are useful in mapping B cell and T cell epitopes of the reference protein. *See, e.g., Geysen et al., Proc. Natl. Acad. Sci. USA* 81: 3998-4002 (1984) and U.S. Patents 4,708,871 and 5,595,915, the disclosures of which are incorporated herein by reference in their entireties. Because the fragment need not itself be immunogenic, part of an immunodominant epitope, nor even recognized by  
30 native antibody, to be useful in such epitope mapping, all fragments of at least 6 amino acids of the proteins of the present invention have utility in such a study.

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Fragments of at least 8 contiguous amino acids, often at least 15 contiguous amino acids, are useful as immunogens for raising antibodies that recognize the proteins of the present invention. *See, e.g.*, Lerner, *Nature* 299: 592-596 (1982); Shinnick *et al.*, *Annu. Rev. Microbiol.* 37: 425-46 (1983); Sutcliffe *et al.*, *Science* 219: 660-6 (1983), the disclosures of which are incorporated herein by reference in their entireties. As further described in the above-cited references, virtually all 8-mers, conjugated to a carrier, such as a protein, prove immunogenic, meaning that they are capable of eliciting antibody for the conjugated peptide; accordingly, all fragments of at least 8 amino acids of the proteins of the present invention have utility as immunogens.

10       Fragments of at least 8, 9, 10 or 12 contiguous amino acids are also useful as competitive inhibitors of binding of the entire protein, or a portion thereof, to antibodies (as in epitope mapping), and to natural binding partners, such as subunits in a multimeric complex or to receptors or ligands of the subject protein; this competitive inhibition permits identification and separation of molecules that bind specifically to the protein of interest, U.S. Patents 5,539,084 and 5,783,674, incorporated herein by reference in their entireties.

20       The protein, or protein fragment, of the present invention is thus at least 6 amino acids in length, typically at least 8, 9, 10 or 12 amino acids in length, and often at least 15 amino acids in length. Often, the protein of the present invention, or fragment thereof, is at least 20 amino acids in length, even 25 amino acids, 30 amino acids, 35 amino acids, or 50 amino acids or more in length. Of course, larger fragments having at least 75 amino acids, 100 amino acids, or even 150 amino acids are also useful, and at times preferred.

25       One having ordinary skill in the art can produce fragments of a polypeptide by truncating the nucleic acid molecule, *e.g.*, a CSNA, encoding the polypeptide and then expressing it recombinantly. Alternatively, one can produce a fragment by chemically synthesizing a portion of the full-length polypeptide. One may also produce a fragment by enzymatically cleaving either a recombinant polypeptide or an isolated naturally-occurring polypeptide. Methods of producing polypeptide fragments are well-known in the art. *See, e.g.*, Sambrook (1989), *supra*; Sambrook (2001), *supra*; Ausubel (1992), *supra*; and Ausubel (1999), *supra*. In one embodiment, a polypeptide comprising only a fragment of polypeptide of the invention, preferably a CSP, may be produced by

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chemical or enzymatic cleavage of a polypeptide. In a preferred embodiment, a polypeptide fragment is produced by expressing a nucleic acid molecule encoding a fragment of the polypeptide, preferably a CSP, in a host cell.

By "polypeptides" as used herein it is also meant to be inclusive of mutants,  
5 fusion proteins, homologous proteins and allelic variants of the polypeptides specifically exemplified.

A mutant protein, or mutein, may have the same or different properties compared to a naturally-occurring polypeptide and comprises at least one amino acid insertion, duplication, deletion, rearrangement or substitution compared to the amino acid sequence  
10 of a native protein. Small deletions and insertions can often be found that do not alter the function of the protein. In one embodiment, the mutein may or may not be colon-specific. In a preferred embodiment, the mutein is colon-specific. In a preferred embodiment, the mutein is a polypeptide that comprises at least one amino acid insertion, duplication, deletion, rearrangement or substitution compared to the amino acid sequence  
15 of SEQ ID NO: 101 through 176. In a more preferred embodiment, the mutein is one that exhibits at least 50% sequence identity, more preferably at least 60% sequence identity, even more preferably at least 70%, yet more preferably at least 80% sequence identity to a CSP comprising an amino acid sequence of SEQ ID NO: 101 through 176. In yet a more preferred embodiment, the mutein exhibits at least 85%, more preferably 90%, even  
20 more preferably 95% or 96%, and yet more preferably at least 97%, 98%, 99% or 99.5% sequence identity to a CSP comprising an amino acid sequence of SEQ ID NO: 101 through 176.

A mutein may be produced by isolation from a naturally-occurring mutant cell, tissue or organism. A mutein may be produced by isolation from a cell, tissue or  
25 organism that has been experimentally mutagenized. Alternatively, a mutein may be produced by chemical manipulation of a polypeptide, such as by altering the amino acid residue to another amino acid residue using synthetic or semi-synthetic chemical techniques. In a preferred embodiment, a mutein may be produced from a host cell comprising an altered nucleic acid molecule compared to the naturally-occurring nucleic  
30 acid molecule. For instance, one may produce a mutein of a polypeptide by introducing one or more mutations into a nucleic acid sequence of the invention and then expressing it recombinantly. These mutations may be targeted, in which particular encoded amino

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acids are altered, or may be untargeted, in which random encoded amino acids within the polypeptide are altered. Muteins with random amino acid alterations can be screened for a particular biological activity or property, particularly whether the polypeptide is colon-specific, as described below. Multiple random mutations can be introduced into the

5 gene by methods well-known to the art, *e.g.*, by error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis and site-specific mutagenesis. Methods of producing muteins with targeted or random amino acid alterations are well-known in the art. *See, e.g.*,

10 Sambrook (1989), *supra*; Sambrook (2001), *supra*; Ausubel (1992), *supra*; and Ausubel (1999), U.S. Patent 5,223,408, and the references discussed *supra*, each herein incorporated by reference.

By "polypeptide" as used herein it is also meant to be inclusive of polypeptides homologous to those polypeptides exemplified herein. In a preferred embodiment, the

15 polypeptide is homologous to a CSP. In an even more preferred embodiment, the polypeptide is homologous to a CSP selected from the group having an amino acid sequence of SEQ ID NO: 101 through 176. In a preferred embodiment, the homologous polypeptide is one that exhibits significant sequence identity to a CSP. In a more preferred embodiment, the polypeptide is one that exhibits significant sequence identity

20 to an comprising an amino acid sequence of SEQ ID NO: 101 through 176. In an even more preferred embodiment, the homologous polypeptide is one that exhibits at least 50% sequence identity, more preferably at least 60% sequence identity, even more preferably at least 70%, yet more preferably at least 80% sequence identity to a CSP comprising an amino acid sequence of SEQ ID NO: 101 through 176. In a yet more

25 preferred embodiment, the homologous polypeptide is one that exhibits at least 85%, more preferably 90%, even more preferably 95% or 96%, and yet more preferably at least 97% or 98% sequence identity to a CSP comprising an amino acid sequence of SEQ ID NO: 101 through 176. In another preferred embodiment, the homologous polypeptide is one that exhibits at least 99%, more preferably 99.5%, even more preferably 99.6%,

30 99.7%, 99.8% or 99.9% sequence identity to a CSP comprising an amino acid sequence of SEQ ID NO: 101 through 176. In a preferred embodiment, the amino acid substitutions are conservative amino acid substitutions as discussed above.

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In another embodiment, the homologous polypeptide is one that is encoded by a nucleic acid molecule that selectively hybridizes to a CSNA. In a preferred embodiment, the homologous polypeptide is encoded by a nucleic acid molecule that hybridizes to a CSNA under low stringency, moderate stringency or high stringency conditions, as defined herein. In a more preferred embodiment, the CSNA is selected from the group consisting of SEQ ID NO: 1 through 100. In another preferred embodiment, the homologous polypeptide is encoded by a nucleic acid molecule that hybridizes to a nucleic acid molecule that encodes a CSP under low stringency, moderate stringency or high stringency conditions, as defined herein. In a more preferred embodiment, the CSP is selected from the group consisting of SEQ ID NO: 101 through 176.

The homologous polypeptide may be a naturally-occurring one that is derived from another species, especially one derived from another primate, such as chimpanzee, gorilla, rhesus macaque, baboon or gorilla, wherein the homologous polypeptide comprises an amino acid sequence that exhibits significant sequence identity to that of SEQ ID NO: 101 through 176. The homologous polypeptide may also be a naturally-occurring polypeptide from a human, when the CSP is a member of a family of polypeptides. The homologous polypeptide may also be a naturally-occurring polypeptide derived from a non-primate, mammalian species, including without limitation, domesticated species, *e.g.*, dog, cat, mouse, rat, rabbit, guinea pig, hamster, cow, horse, goat or pig. The homologous polypeptide may also be a naturally-occurring polypeptide derived from a non-mammalian species, such as birds or reptiles. The naturally-occurring homologous protein may be isolated directly from humans or other species. Alternatively, the nucleic acid molecule encoding the naturally-occurring homologous polypeptide may be isolated and used to express the homologous polypeptide recombinantly. In another embodiment, the homologous polypeptide may be one that is experimentally produced by random mutation of a nucleic acid molecule and subsequent expression of the nucleic acid molecule. In another embodiment, the homologous polypeptide may be one that is experimentally produced by directed mutation of one or more codons to alter the encoded amino acid of a CSP. Further, the homologous protein may or may not encode polypeptide that is a CSP. However, in a preferred embodiment, the homologous polypeptide encodes a polypeptide that is a CSP.

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Relatedness of proteins can also be characterized using a second functional test, the ability of a first protein competitively to inhibit the binding of a second protein to an antibody. It is, therefore, another aspect of the present invention to provide isolated proteins not only identical in sequence to those described with particularity herein, but  
5 also to provide isolated proteins (“cross-reactive proteins”) that competitively inhibit the binding of antibodies to all or to a portion of various of the isolated polypeptides of the present invention. Such competitive inhibition can readily be determined using immunoassays well-known in the art.

As discussed above, single nucleotide polymorphisms (SNPs) occur frequently in  
10 eukaryotic genomes, and the sequence determined from one individual of a species may differ from other allelic forms present within the population. Thus, by “polypeptide” as used herein it is also meant to be inclusive of polypeptides encoded by an allelic variant of a nucleic acid molecule encoding a CSP. In a preferred embodiment, the polypeptide is encoded by an allelic variant of a gene that encodes a polypeptide having the amino  
15 acid sequence selected from the group consisting of SEQ ID NO: 101 through 176. In a yet more preferred embodiment, the polypeptide is encoded by an allelic variant of a gene that has the nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through 100.

In another embodiment, the invention provides polypeptides which comprise  
20 derivatives of a polypeptide encoded by a nucleic acid molecule according to the instant invention. In a preferred embodiment, the polypeptide is a CSP. In a preferred embodiment, the polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 101 through 176, or is a mutein, allelic variant, homologous protein or fragment thereof. In a preferred embodiment, the derivative has been  
25 acetylated, carboxylated, phosphorylated, glycosylated or ubiquitinated. In another preferred embodiment, the derivative has been labeled with, *e.g.*, radioactive isotopes such as  $^{125}\text{I}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ , and  $^3\text{H}$ . In another preferred embodiment, the derivative has been labeled with fluorophores, chemiluminescent agents, enzymes, and antiligands that can serve as specific binding pair members for a labeled ligand.

30 Polypeptide modifications are well-known to those of skill and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of



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glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as, for instance Creighton, Protein Structure and Molecular Properties, 2nd ed., W. H. Freeman and Company (1993). Many detailed reviews are available on this subject, such as, for example, those provided by Wold, in Johnson (ed.),  
5 Posttranslational Covalent Modification of Proteins, pgs. 1-12, Academic Press (1983); Seifter *et al.*, *Meth. Enzymol.* 182: 626-646 (1990) and Rattan *et al.*, *Ann. N.Y. Acad. Sci.* 663: 48-62 (1992).

It will be appreciated, as is well-known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of  
10 ubiquitination, and they may be circular, with or without branching, generally as a result of posttranslation events, including natural processing event and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translation natural process and by entirely synthetic methods, as well. Modifications can occur anywhere in a polypeptide,  
15 including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention, as well. For instance, the amino terminal residue of polypeptides made in *E. coli*, prior to proteolytic  
20 processing, almost invariably will be N-formylmethionine.

Useful post-synthetic (and post-translational) modifications include conjugation to detectable labels, such as fluorophores. A wide variety of amine-reactive and thiol-reactive fluorophore derivatives have been synthesized that react under nondenaturing conditions with N-terminal amino groups and epsilon amino groups of lysine residues, on  
25 the one hand, and with free thiol groups of cysteine residues, on the other.

Kits are available commercially that permit conjugation of proteins to a variety of amine-reactive or thiol-reactive fluorophores: Molecular Probes, Inc. (Eugene, OR, USA), *e.g.*, offers kits for conjugating proteins to Alexa Fluor 350, Alexa Fluor 430, Fluorescein-EX, Alexa Fluor 488, Oregon Green 488, Alexa Fluor 532, Alexa Fluor 546,  
30 Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, and Texas Red-X.

A wide variety of other amine-reactive and thiol-reactive fluorophores are available commercially (Molecular Probes, Inc., Eugene, OR, USA), including Alexa

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Fluor® 350, Alexa Fluor® 488, Alexa Fluor® 532, Alexa Fluor® 546, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 647 (monoclonal antibody labeling kits available from Molecular Probes, Inc., Eugene, OR, USA), BODIPY dyes, such as BODIPY 493/503, BODIPY FL, BODIPY R6G, BODIPY 530/550, BODIPY TMR, BODIPY 558/568, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589, BODIPY 581/591, BODIPY TR, BODIPY 630/650, BODIPY 650/665, Cascade Blue, Cascade Yellow, Dansyl, lissamine rhodamine B, Marina Blue, Oregon Green 488, Oregon Green 514, Pacific Blue, rhodamine 6G, rhodamine green, rhodamine red, tetramethylrhodamine, Texas Red (available from Molecular Probes, Inc., Eugene, OR, USA).

The polypeptides of the present invention can also be conjugated to fluorophores, other proteins, and other macromolecules, using bifunctional linking reagents. Common homobifunctional reagents include, *e.g.*, APG, AEDP, BASED, BMB, BMDB, BMH, BMOE, BM[PEO]3, BM[PEO]4, BS3, BSOCOES, DFDNB, DMA, DMP, DMS, DPDPB, DSG, DSP (Lomant's Reagent), DSS, DST, DTBP, DTME, DTSSP, EGS, HBVS, Sulfo-BSOCOES, Sulfo-DST, Sulfo-EGS (all available from Pierce, Rockford, IL, USA); common heterobifunctional cross-linkers include ABH, AMAS, ANB-NOS, APDP, ASBA, BMPPA, BMPH, BMPS, EDC, EMCA, EMCH, EMCS, KMUA, KMHU, GMBS, LC-SMCC, LC-SPDP, MBS, M2C2H, MPBH, MSA, NHS-ASA, PDPH, PMPI, SADP, SAED, SAND, SANPAH, SASD, SATP, SBAP, SFAD, SIA, SIAB, SMCC, SMPB, SMPH, SMPT, SPDP, Sulfo-EMCS, Sulfo-GMBS, Sulfo-HSAB, Sulfo-KMUS, Sulfo-LC-SPDP, Sulfo-MBS, Sulfo-NHS-LC-ASA, Sulfo-SADP, Sulfo-SANPAH, Sulfo-SIAB, Sulfo-SMCC, Sulfo-SMPB, Sulfo-LC-SMPT, SVSB, TFCS (all available Pierce, Rockford, IL, USA).

The polypeptides, fragments, and fusion proteins of the present invention can be conjugated, using such cross-linking reagents, to fluorophores that are not amine- or thiol-reactive. Other labels that usefully can be conjugated to the polypeptides, fragments, and fusion proteins of the present invention include radioactive labels, echosonographic contrast reagents, and MRI contrast agents.

The polypeptides, fragments, and fusion proteins of the present invention can also usefully be conjugated using cross-linking agents to carrier proteins, such as KLH, bovine thyroglobulin, and even bovine serum albumin (BSA), to increase immunogenicity for raising anti-CSP antibodies.

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The polypeptides, fragments, and fusion proteins of the present invention can also usefully be conjugated to polyethylene glycol (PEG); PEGylation increases the serum half-life of proteins administered intravenously for replacement therapy. Delgado *et al.*, *Crit. Rev. Ther. Drug Carrier Syst.* 9(3-4): 249-304 (1992); Scott *et al.*, *Curr. Pharm. Des.* 4(6): 423-38 (1998); DeSantis *et al.*, *Curr. Opin. Biotechnol.* 10(4): 324-30 (1999), incorporated herein by reference in their entireties. PEG monomers can be attached to the protein directly or through a linker, with PEGylation using PEG monomers activated with tresyl chloride (2,2,2-trifluoroethanesulphonyl chloride) permitting direct attachment under mild conditions.

10 In yet another embodiment, the invention provides analogs of a polypeptide encoded by a nucleic acid molecule according to the instant invention. In a preferred embodiment, the polypeptide is a CSP. In a more preferred embodiment, the analog is derived from a polypeptide having part or all of the amino acid sequence of SEQ ID NO: 101 through 176. In a preferred embodiment, the analog is one that comprises one or  
15 more substitutions of non-natural amino acids or non-native inter-residue bonds compared to the naturally-occurring polypeptide. In general, the non-peptide analog is structurally similar to a CSP, but one or more peptide linkages is replaced by a linkage selected from the group consisting of --CH<sub>2</sub>NH--, --CH<sub>2</sub>S--, --CH<sub>2</sub>-CH<sub>2</sub>--,  
--CH=CH--(cis and trans), --COCH<sub>2</sub>--, --CH(OH)CH<sub>2</sub>-- and --CH<sub>2</sub>SO--. In another  
20 embodiment, the non-peptide analog comprises substitution of one or more amino acids of a CSP with a D-amino acid of the same type or other non-natural amino acid in order to generate more stable peptides. D-amino acids can readily be incorporated during chemical peptide synthesis: peptides assembled from D-amino acids are more resistant to proteolytic attack; incorporation of D-amino acids can also be used to confer specific  
25 three-dimensional conformations on the peptide. Other amino acid analogues commonly added during chemical synthesis include ornithine, norleucine, phosphorylated amino acids (typically phosphoserine, phosphothreonine, phosphotyrosine), L-malonyltyrosine, a non-hydrolyzable analog of phosphotyrosine (*see, e.g.*, Kole *et al.*, *Biochem. Biophys. Res. Com.* 209: 817-821 (1995)), and various halogenated phenylalanine derivatives.

30 Non-natural amino acids can be incorporated during solid phase chemical synthesis or by recombinant techniques, although the former is typically more common. Solid phase chemical synthesis of peptides is well established in the art. Procedures are

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described, inter alia, in Chan *et al.* (eds.), Fmoc Solid Phase Peptide Synthesis: A Practical Approach (Practical Approach Series), Oxford Univ. Press (March 2000); Jones, Amino Acid and Peptide Synthesis (Oxford Chemistry Primers, No 7), Oxford Univ. Press (1992); and Bodanszky, Principles of Peptide Synthesis (Springer Laboratory), Springer Verlag (1993); the disclosures of which are incorporated herein by reference in their entireties.

Amino acid analogues having detectable labels are also usefully incorporated during synthesis to provide derivatives and analogs. Biotin, for example can be added using biotinoyl-(9-fluorenylmethoxycarbonyl)-L-lysine (Fmoc biocytin) (Molecular Probes, Eugene, OR, USA). Biotin can also be added enzymatically by incorporation into a fusion protein of a *E. coli* BirA substrate peptide. The Fmoc and *t*BOC derivatives of dabcyL-L-lysine (Molecular Probes, Inc., Eugene, OR, USA) can be used to incorporate the dabcyL chromophore at selected sites in the peptide sequence during synthesis. The aminonaphthalene derivative EDANS, the most common fluorophore for pairing with the dabcyL quencher in fluorescence resonance energy transfer (FRET) systems, can be introduced during automated synthesis of peptides by using EDANS-Fmoc-L-glutamic acid or the corresponding *t*BOC derivative (both from Molecular Probes, Inc., Eugene, OR, USA). Tetramethylrhodamine fluorophores can be incorporated during automated Fmoc synthesis of peptides using (Fmoc)-TMR-L-lysine (Molecular Probes, Inc. Eugene, OR, USA).

Other useful amino acid analogues that can be incorporated during chemical synthesis include aspartic acid, glutamic acid, lysine, and tyrosine analogues having allyl side-chain protection (Applied Biosystems, Inc., Foster City, CA, USA); the allyl side chain permits synthesis of cyclic, branched-chain, sulfonated, glycosylated, and phosphorylated peptides.

A large number of other Fmoc-protected non-natural amino acid analogues capable of incorporation during chemical synthesis are available commercially, including, *e.g.*, Fmoc-2-aminobicyclo[2.2.1]heptane-2-carboxylic acid, Fmoc-3-endo-aminobicyclo[2.2.1]heptane-2-endo-carboxylic acid, Fmoc-3-exo-aminobicyclo[2.2.1]heptane-2-exo-carboxylic acid, Fmoc-3-endo-amino-bicyclo[2.2.1]hept-5-ene-2-endo-carboxylic acid, Fmoc-3-exo-amino-bicyclo[2.2.1]hept-5-ene-2-exo-carboxylic acid, Fmoc-cis-2-amino-1-cyclohexanecarboxylic acid, Fmoc-

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trans-2-amino-1-cyclohexanecarboxylic acid, Fmoc-1-amino-1-cyclopentanecarboxylic acid, Fmoc-cis-2-amino-1-cyclopentanecarboxylic acid, Fmoc-1-amino-1-cyclopropanecarboxylic acid, Fmoc-D-2-amino-4-(ethylthio)butyric acid, Fmoc-L-2-amino-4-(ethylthio)butyric acid, Fmoc-L-buthionine, Fmoc-S-methyl-L-Cysteine, Fmoc-  
 5 2-aminobenzoic acid (anthranillic acid), Fmoc-3-aminobenzoic acid, Fmoc-4-aminobenzoic acid, Fmoc-2-aminobenzophenone-2'-carboxylic acid, Fmoc-N-(4-aminobenzoyl)- $\beta$ -alanine, Fmoc-2-amino-4,5-dimethoxybenzoic acid, Fmoc-4-aminohippuric acid, Fmoc-2-amino-3-hydroxybenzoic acid, Fmoc-2-amino-5-hydroxybenzoic acid, Fmoc-3-amino-4-hydroxybenzoic acid, Fmoc-4-amino-3-  
 10 hydroxybenzoic acid, Fmoc-4-amino-2-hydroxybenzoic acid, Fmoc-5-amino-2-hydroxybenzoic acid, Fmoc-2-amino-3-methoxybenzoic acid, Fmoc-4-amino-3-methoxybenzoic acid, Fmoc-2-amino-3-methylbenzoic acid, Fmoc-2-amino-5-methylbenzoic acid, Fmoc-2-amino-6-methylbenzoic acid, Fmoc-3-amino-2-methylbenzoic acid, Fmoc-3-amino-4-methylbenzoic acid, Fmoc-4-amino-3-  
 15 methylbenzoic acid, Fmoc-3-amino-2-naphtoic acid, Fmoc-D,L-3-amino-3-phenylpropionic acid, Fmoc-L-Methyldopa, Fmoc-2-amino-4,6-dimethyl-3-pyridinecarboxylic acid, Fmoc-D,L-amino-2-thiophenacetic acid, Fmoc-4-(carboxymethyl)piperazine, Fmoc-4-carboxypiperazine, Fmoc-4-(carboxymethyl)homopiperazine, Fmoc-4-phenyl-4-piperidinecarboxylic acid, Fmoc-L-  
 20 1,2,3,4-tetrahydronorharman-3-carboxylic acid, Fmoc-L-thiazolidine-4-carboxylic acid, all available from The Peptide Laboratory (Richmond, CA, USA).

Non-natural residues can also be added biosynthetically by engineering a suppressor tRNA, typically one that recognizes the UAG stop codon, by chemical aminoacylation with the desired unnatural amino acid. Conventional site-directed  
 25 mutagenesis is used to introduce the chosen stop codon UAG at the site of interest in the protein gene. When the acylated suppressor tRNA and the mutant gene are combined in an *in vitro* transcription/translation system, the unnatural amino acid is incorporated in response to the UAG codon to give a protein containing that amino acid at the specified position. Liu *et al.*, *Proc. Natl Acad. Sci. USA* 96(9): 4780-5 (1999); Wang *et al.*,  
 30 *Science* 292(5516): 498-500 (2001).

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*Fusion Proteins*

The present invention further provides fusions of each of the polypeptides and fragments of the present invention to heterologous polypeptides. In a preferred embodiment, the polypeptide is a CSP. In a more preferred embodiment, the polypeptide  
5 that is fused to the heterologous polypeptide comprises part or all of the amino acid sequence of SEQ ID NO: 101 through 176, or is a mutein, homologous polypeptide, analog or derivative thereof. In an even more preferred embodiment, the nucleic acid molecule encoding the fusion protein comprises all or part of the nucleic acid sequence of SEQ ID NO: 1 through 100, or comprises all or part of a nucleic acid sequence that  
10 selectively hybridizes or is homologous to a nucleic acid molecule comprising a nucleic acid sequence of SEQ ID NO: 1 through 100.

The fusion proteins of the present invention will include at least one fragment of the protein of the present invention, which fragment is at least 6, typically at least 8, often at least 15, and usefully at least 16, 17, 18, 19, or 20 amino acids long. The fragment of  
15 the protein of the present to be included in the fusion can usefully be at least 25 amino acids long, at least 50 amino acids long, and can be at least 75, 100, or even 150 amino acids long. Fusions that include the entirety of the proteins of the present invention have particular utility.

The heterologous polypeptide included within the fusion protein of the present  
20 invention is at least 6 amino acids in length, often at least 8 amino acids in length, and usefully at least 15, 20, and 25 amino acids in length. Fusions that include larger polypeptides, such as the IgG Fc region, and even entire proteins (such as GFP chromophore-containing proteins) are particular useful.

As described above in the description of vectors and expression vectors of the  
25 present invention, which discussion is incorporated here by reference in its entirety, heterologous polypeptides to be included in the fusion proteins of the present invention can usefully include those designed to facilitate purification and/or visualization of recombinantly-expressed proteins. *See, e.g., Ausubel, Chapter 16, (1992), supra.* Although purification tags can also be incorporated into fusions that are chemically  
30 synthesized, chemical synthesis typically provides sufficient purity that further purification by HPLC suffices; however, visualization tags as above described retain their utility even when the protein is produced by chemical synthesis, and when so

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included render the fusion proteins of the present invention useful as directly detectable markers of the presence of a polypeptide of the invention.

As also discussed above, heterologous polypeptides to be included in the fusion proteins of the present invention can usefully include those that facilitate secretion of recombinantly expressed proteins — into the periplasmic space or extracellular milieu for prokaryotic hosts, into the culture medium for eukaryotic cells — through incorporation of secretion signals and/or leader sequences. For example, a His<sup>6</sup> tagged protein can be purified on a Ni affinity column and a GST fusion protein can be purified on a glutathione affinity column. Similarly, a fusion protein comprising the Fc domain of IgG can be purified on a Protein A or Protein G column and a fusion protein comprising an epitope tag such as myc can be purified using an immunoaffinity column containing an anti-c-myc antibody. It is preferable that the epitope tag be separated from the protein encoded by the essential gene by an enzymatic cleavage site that can be cleaved after purification. See also the discussion of nucleic acid molecules encoding fusion proteins that may be expressed on the surface of a cell.

Other useful protein fusions of the present invention include those that permit use of the protein of the present invention as bait in a yeast two-hybrid system. See Bartel *et al.* (eds.), The Yeast Two-Hybrid System, Oxford University Press (1997); Zhu *et al.*, Yeast Hybrid Technologies, Eaton Publishing (2000); Fields *et al.*, *Trends Genet.* 10(8): 286-92 (1994); Mendelsohn *et al.*, *Curr. Opin. Biotechnol.* 5(5): 482-6 (1994); Luban *et al.*, *Curr. Opin. Biotechnol.* 6(1): 59-64 (1995); Allen *et al.*, *Trends Biochem. Sci.* 20(12): 511-6 (1995); Drees, *Curr. Opin. Chem. Biol.* 3(1): 64-70 (1999); Topcu *et al.*, *Pharm. Res.* 17(9): 1049-55 (2000); Fashena *et al.*, *Gene* 250(1-2): 1-14 (2000); ; Colas *et al.*, (1996) Genetic selection of peptide aptamers that recognize and inhibit cyclin-dependent kinase 2. *Nature* 380, 548-550; Norman, T. *et al.*, (1999) Genetic selection of peptide inhibitors of biological pathways. *Science* 285, 591-595, Fabbrizio *et al.*, (1999) Inhibition of mammalian cell proliferation by genetically selected peptide aptamers that functionally antagonize E2F activity. *Oncogene* 18, 4357-4363; Xu *et al.*, (1997) Cells that register logical relationships among proteins. *Proc Natl Acad Sci U S A.* 94, 12473-12478; Yang, *et al.*, (1995) Protein-peptide interactions analyzed with the yeast two-hybrid system. *Nuc. Acids Res.* 23, 1152-1156; Kolonin *et al.*, (1998) Targeting cyclin-dependent kinases in *Drosophila* with peptide aptamers. *Proc Natl Acad Sci U S A* 95,

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14266-14271; Cohen *et al.*, (1998) An artificial cell-cycle inhibitor isolated from a combinatorial library. *Proc Natl Acad Sci U S A* 95, 14272-14277; Uetz, P.; Giot, L.; al, e.; Fields, S.; Rothberg, J. M. (2000) A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. *Nature* 403, 623-627; Ito, *et al.*, (2001) A  
5 comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc Natl Acad Sci U S A* 98, 4569-4574, the disclosures of which are incorporated herein by reference in their entireties. Typically, such fusion is to either *E. coli* LexA or yeast GAL4 DNA binding domains. Related bait plasmids are available that express the bait fused to a nuclear localization signal.

10 Other useful fusion proteins include those that permit display of the encoded protein on the surface of a phage or cell, fusions to intrinsically fluorescent proteins, such as green fluorescent protein (GFP), and fusions to the IgG Fc region, as described above, which discussion is incorporated here by reference in its entirety.

The polypeptides and fragments of the present invention can also usefully be  
15 fused to protein toxins, such as *Pseudomonas* exotoxin A, *diphtheria* toxin, *shiga* toxin A, *anthrax* toxin lethal factor, ricin, in order to effect ablation of cells that bind or take up the proteins of the present invention.

Fusion partners include, *inter alia*, *myc*, hemagglutinin (HA), GST, immunoglobulins,  $\beta$ -galactosidase, biotin *trpE*, protein A,  $\beta$ -lactamase,  $\alpha$ -amylase,  
20 maltose binding protein, alcohol dehydrogenase, polyhistidine (for example, six histidine at the amino and/or carboxyl terminus of the polypeptide), *lacZ*, green fluorescent protein (GFP), yeast  $\alpha$ -mating factor, GAL4 transcription activation or DNA binding domain, luciferase, and serum proteins such as ovalbumin, albumin and the constant domain of IgG. See, e.g., Ausubel (1992), *supra* and Ausubel (1999), *supra*. Fusion proteins may  
25 also contain sites for specific enzymatic cleavage, such as a site that is recognized by enzymes such as Factor XIII, trypsin, pepsin, or any other enzyme known in the art. Fusion proteins will typically be made by either recombinant nucleic acid methods, as described above, chemically synthesized using techniques well-known in the art (e.g., a Merrifield synthesis), or produced by chemical cross-linking.

30 Another advantage of fusion proteins is that the epitope tag can be used to bind the fusion protein to a plate or column through an affinity linkage for screening binding proteins or other molecules that bind to the CSP.



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As further described below, the isolated polypeptides, muteins, fusion proteins, homologous proteins or allelic variants of the present invention can readily be used as specific immunogens to raise antibodies that specifically recognize CSPs, their allelic variants and homologues. The antibodies, in turn, can be used, *inter alia*, specifically to  
5 assay for the polypeptides of the present invention, particularly CSPs, *e.g.* by ELISA for detection of protein fluid samples, such as serum, by immunohistochemistry or laser scanning cytometry, for detection of protein in tissue samples, or by flow cytometry, for detection of intracellular protein in cell suspensions, for specific antibody-mediated isolation and/or purification of CSPs, as for example by immunoprecipitation, and for use  
10 as specific agonists or antagonists of CSPs.

One may determine whether polypeptides including muteins, fusion proteins, homologous proteins or allelic variants are functional by methods known in the art. For instance, residues that are tolerant of change while retaining function can be identified by altering the protein at known residues using methods known in the art, such as alanine  
15 scanning mutagenesis, Cunningham *et al.*, *Science* 244(4908): 1081-5 (1989); transposon linker scanning mutagenesis, Chen *et al.*, *Gene* 263(1-2): 39-48 (2001); combinations of homolog- and alanine-scanning mutagenesis, Jin *et al.*, *J. Mol. Biol.* 226(3): 851-65 (1992); combinatorial alanine scanning, Weiss *et al.*, *Proc. Natl. Acad. Sci USA* 97(16): 8950-4 (2000), followed by functional assay. Transposon linker scanning kits are  
20 available commercially (New England Biolabs, Beverly, MA, USA, catalog. no. E7-102S; EZ::TN™ In-Frame Linker Insertion Kit, catalogue no. EZI04KN, Epicentre Technologies Corporation, Madison, WI, USA).

Purification of the polypeptides including fragments, homologous polypeptides, muteins, analogs, derivatives and fusion proteins is well-known and within the skill of  
25 one having ordinary skill in the art. *See, e.g.*, Scopes, Protein Purification, 2d ed. (1987). Purification of recombinantly expressed polypeptides is described above. Purification of chemically-synthesized peptides can readily be effected, *e.g.*, by HPLC.

Accordingly, it is an aspect of the present invention to provide the isolated proteins of the present invention in pure or substantially pure form in the presence of  
30 absence of a stabilizing agent. Stabilizing agents include both proteinaceous or non-proteinaceous material and are well-known in the art. Stabilizing agents, such as albumin and polyethylene glycol (PEG) are known and are commercially available.

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Although high levels of purity are preferred when the isolated proteins of the present invention are used as therapeutic agents, such as in vaccines and as replacement therapy, the isolated proteins of the present invention are also useful at lower purity. For example, partially purified proteins of the present invention can be used as immunogens  
5 to raise antibodies in laboratory animals.

In preferred embodiments, the purified and substantially purified proteins of the present invention are in compositions that lack detectable ampholytes, acrylamide monomers, bis-acrylamide monomers, and polyacrylamide.

The polypeptides, fragments, analogs, derivatives and fusions of the present  
10 invention can usefully be attached to a substrate. The substrate can be porous or solid, planar or non-planar; the bond can be covalent or noncovalent.

For example, the polypeptides, fragments, analogs, derivatives and fusions of the present invention can usefully be bound to a porous substrate, commonly a membrane, typically comprising nitrocellulose, polyvinylidene fluoride (PVDF), or cationically  
15 derivatized, hydrophilic PVDF; so bound, the proteins, fragments, and fusions of the present invention can be used to detect and quantify antibodies, *e.g.* in serum, that bind specifically to the immobilized protein of the present invention.

As another example, the polypeptides, fragments, analogs, derivatives and fusions of the present invention can usefully be bound to a substantially nonporous substrate,  
20 such as plastic, to detect and quantify antibodies, *e.g.* in serum, that bind specifically to the immobilized protein of the present invention. Such plastics include polymethylacrylic, polyethylene, polypropylene, polyacrylate, polymethylmethacrylate, polyvinylchloride, polytetrafluoroethylene, polystyrene, polycarbonate, polyacetal, polysulfone, celluloseacetate, cellulosenitrate, nitrocellulose, or mixtures thereof; when  
25 the assay is performed in a standard microtiter dish, the plastic is typically polystyrene.

The polypeptides, fragments, analogs, derivatives and fusions of the present invention can also be attached to a substrate suitable for use as a surface enhanced laser desorption ionization source; so attached, the protein, fragment, or fusion of the present invention is useful for binding and then detecting secondary proteins that bind with  
30 sufficient affinity or avidity to the surface-bound protein to indicate biologic interaction there between. The proteins, fragments, and fusions of the present invention can also be attached to a substrate suitable for use in surface plasmon resonance detection; so

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attached, the protein, fragment, or fusion of the present invention is useful for binding and then detecting secondary proteins that bind with sufficient affinity or avidity to the surface-bound protein to indicate biological interaction there between.

### Antibodies

5           In another aspect, the invention provides antibodies, including fragments and derivatives thereof, that bind specifically to polypeptides encoded by the nucleic acid molecules of the invention, as well as antibodies that bind to fragments, muteins, derivatives and analogs of the polypeptides. In a preferred embodiment, the antibodies are specific for a polypeptide that is a CSP, or a fragment, mutein, derivative, analog or  
10 fusion protein thereof. In a more preferred embodiment, the antibodies are specific for a polypeptide that comprises SEQ ID NO: 101 through 176, or a fragment, mutein, derivative, analog or fusion protein thereof.

          The antibodies of the present invention can be specific for linear epitopes, discontinuous epitopes, or conformational epitopes of such proteins or protein fragments,  
15 either as present on the protein in its native conformation or, in some cases, as present on the proteins as denatured, as, *e.g.*, by solubilization in SDS. New epitopes may be also due to a difference in post translational modifications (PTMs) in disease versus normal tissue. For example, a particular site on a CSP may be glycosylated in cancerous cells, but not glycosylated in normal cells or *visa versa*. In addition, alternative splice forms  
20 of a CSP may be indicative of cancer. Differential degradation of the C or N-terminus of a CSP may also be a marker or target for anticancer therapy. For example, a CSP may be N-terminal degraded in cancer cells exposing new epitopes to which antibodies may selectively bind for diagnostic or therapeutic uses.

          As is well-known in the art, the degree to which an antibody can discriminate as  
25 among molecular species in a mixture will depend, in part, upon the conformational relatedness of the species in the mixture; typically, the antibodies of the present invention will discriminate over adventitious binding to non-CSP polypeptides by at least 2-fold, more typically by at least 5-fold, typically by more than 10-fold, 25-fold, 50-fold, 75-fold, and often by more than 100-fold, and on occasion by more than 500-fold or 1000-  
30 fold. When used to detect the proteins or protein fragments of the present invention, the antibody of the present invention is sufficiently specific when it can be used to determine

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the presence of the protein of the present invention in samples derived from human colon.

Typically, the affinity or avidity of an antibody (or antibody multimer, as in the case of an IgM pentamer) of the present invention for a protein or protein fragment of the present invention will be at least about  $1 \times 10^{-6}$  molar (M), typically at least about  $5 \times 10^{-7}$  M,  $1 \times 10^{-7}$  M, with affinities and avidities of at least  $1 \times 10^{-8}$  M,  $5 \times 10^{-9}$  M,  $1 \times 10^{-10}$  M and up to  $1 \times 10^{-13}$  M proving especially useful.

The antibodies of the present invention can be naturally-occurring forms, such as IgG, IgM, IgD, IgE, IgY, and IgA, from any avian, reptilian, or mammalian species.

Human antibodies can, but will infrequently, be drawn directly from human donors or human cells. In this case, antibodies to the proteins of the present invention will typically have resulted from fortuitous immunization, such as autoimmune immunization, with the protein or protein fragments of the present invention. Such antibodies will typically, but will not invariably, be polyclonal. In addition, individual polyclonal antibodies may be isolated and cloned to generate monoclonals.

Human antibodies are more frequently obtained using transgenic animals that express human immunoglobulin genes, which transgenic animals can be affirmatively immunized with the protein immunogen of the present invention. Human Ig-transgenic mice capable of producing human antibodies and methods of producing human antibodies therefrom upon specific immunization are described, *inter alia*, in U.S. Patents 6,162,963; 6,150,584; 6,114,598; 6,075,181; 5,939,598; 5,877,397; 5,874,299; 5,814,318; 5,789,650; 5,770,429; 5,661,016; 5,633,425; 5,625,126; 5,569,825; 5,545,807; 5,545,806, and 5,591,669, the disclosures of which are incorporated herein by reference in their entireties. Such antibodies are typically monoclonal, and are typically produced using techniques developed for production of murine antibodies.

Human antibodies are particularly useful, and often preferred, when the antibodies of the present invention are to be administered to human beings as *in vivo* diagnostic or therapeutic agents, since recipient immune response to the administered antibody will often be substantially less than that occasioned by administration of an antibody derived from another species, such as mouse.

IgG, IgM, IgD, IgE, IgY, and IgA antibodies of the present invention can also be obtained from other species, including mammals such as rodents (typically mouse, but

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also rat, guinea pig, and hamster) lagomorphs, typically rabbits, and also larger mammals, such as sheep, goats, cows, and horses, and other egg laying birds or reptiles such as chickens or alligators. For example, avian antibodies may be generated using techniques described in WO 00/29444, published 25 May 2000, the contents of which are hereby incorporated in their entirety. In such cases, as with the transgenic human-antibody-producing non-human mammals, fortuitous immunization is not required, and the non-human mammal is typically affirmatively immunized, according to standard immunization protocols, with the protein or protein fragment of the present invention.

As discussed above, virtually all fragments of 8 or more contiguous amino acids of the proteins of the present invention can be used effectively as immunogens when conjugated to a carrier, typically a protein such as bovine thyroglobulin, keyhole limpet hemocyanin, or bovine serum albumin, conveniently using a bifunctional linker such as those described elsewhere above, which discussion is incorporated by reference here.

Immunogenicity can also be conferred by fusion of the polypeptide and fragments of the present invention to other moieties. For example, peptides of the present invention can be produced by solid phase synthesis on a branched polylysine core matrix; these multiple antigenic peptides (MAPs) provide high purity, increased avidity, accurate chemical definition and improved safety in vaccine development. Tam *et al.*, *Proc. Natl. Acad. Sci. USA* 85: 5409-5413 (1988); Posnett *et al.*, *J. Biol. Chem.* 263: 1719-1725 (1988).

Protocols for immunizing non-human mammals or avian species are well-established in the art. See Harlow *et al.* (eds.), Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory (1998); Coligan *et al.* (eds.), Current Protocols in Immunology, John Wiley & Sons, Inc. (2001); Zola, Monoclonal Antibodies: Preparation and Use of Monoclonal Antibodies and Engineered Antibody Derivatives (Basics: From Background to Bench), Springer Verlag (2000); Gross M, Speck *J.Dtsch. Tierarztl. Wochenschr.* 103: 417-422 (1996), the disclosures of which are incorporated herein by reference. Immunization protocols often include multiple immunizations, either with or without adjuvants such as Freund's complete adjuvant and Freund's incomplete adjuvant, and may include naked DNA immunization (Moss, *Semin. Immunol.* 2: 317-327 (1990)).

Antibodies from non-human mammals and avian species can be polyclonal or monoclonal, with polyclonal antibodies having certain advantages in

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immunohistochemical detection of the proteins of the present invention and monoclonal antibodies having advantages in identifying and distinguishing particular epitopes of the proteins of the present invention. Antibodies from avian species may have particular advantage in detection of the proteins of the present invention, in human serum or tissues

5 (Vikinge et al., *Biosens. Bioelectron.* 13: 1257-1262 (1998).

Following immunization, the antibodies of the present invention can be produced using any art-accepted technique. Such techniques are well-known in the art, Coligan, *supra*; Zola, *supra*; Howard et al. (eds.), Basic Methods in Antibody Production and Characterization, CRC Press (2000); Harlow, *supra*; Davis (ed.), Monoclonal Antibody

10 Protocols, Vol. 45, Humana Press (1995); Delves (ed.), Antibody Production: Essential Techniques, John Wiley & Son Ltd (1997); Kenney, Antibody Solution: An Antibody Methods Manual, Chapman & Hall (1997), incorporated herein by reference in their entirety, and thus need not be detailed here.

Briefly, however, such techniques include, *inter alia*, production of monoclonal

15 antibodies by hybridomas and expression of antibodies or fragments or derivatives thereof from host cells engineered to express immunoglobulin genes or fragments thereof. These two methods of production are not mutually exclusive: genes encoding antibodies specific for the proteins or protein fragments of the present invention can be cloned from hybridomas and thereafter expressed in other host cells. Nor need the two

20 necessarily be performed together: *e.g.*, genes encoding antibodies specific for the proteins and protein fragments of the present invention can be cloned directly from B cells known to be specific for the desired protein, as further described in U.S. Patent 5,627,052, the disclosure of which is incorporated herein by reference in its entirety, or from antibody-displaying phage.

25 Recombinant expression in host cells is particularly useful when fragments or derivatives of the antibodies of the present invention are desired.

Host cells for recombinant production of either whole antibodies, antibody fragments, or antibody derivatives can be prokaryotic or eukaryotic.

Prokaryotic hosts are particularly useful for producing phage displayed antibodies

30 of the present invention.

The technology of phage-displayed antibodies, in which antibody variable region fragments are fused, for example, to the gene III protein (pIII) or gene VIII protein

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(pVIII) for display on the surface of filamentous phage, such as M13, is by now well-established. *See, e.g.,* Sidhu, *Curr. Opin. Biotechnol.* 11(6): 610-6 (2000); Griffiths *et al., Curr. Opin. Biotechnol.* 9(1): 102-8 (1998); Hoogenboom *et al., Immunotechnology*, 4(1): 1-20 (1998); Rader *et al., Current Opinion in Biotechnology* 8: 503-508 (1997);

5 Aujame *et al., Human Antibodies* 8: 155-168 (1997); Hoogenboom, *Trends in Biotechnol.* 15: 62-70 (1997); de Kruif *et al.,* 17: 453-455 (1996); Barbas *et al., Trends in Biotechnol.* 14: 230-234 (1996); Winter *et al., Ann. Rev. Immunol.* 433-455 (1994).

Techniques and protocols required to generate, propagate, screen (pan), and use the antibody fragments from such libraries have recently been compiled. *See, e.g.,* Barbas  
10 (2001), *supra*; Kay, *supra*; Abelson, *supra*, the disclosures of which are incorporated herein by reference in their entirety.

Typically, phage-displayed antibody fragments are scFv fragments or Fab fragments; when desired, full length antibodies can be produced by cloning the variable regions from the displaying phage into a complete antibody and expressing the full length  
15 antibody in a further prokaryotic or a eukaryotic host cell.

Eukaryotic cells are also useful for expression of the antibodies, antibody fragments, and antibody derivatives of the present invention.

For example, antibody fragments of the present invention can be produced in *Pichia pastoris* and in *Saccharomyces cerevisiae*. *See, e.g.,* Takahashi *et al., Biosci.*  
20 *Biotechnol. Biochem.* 64(10): 2138-44 (2000); Freyre *et al., J. Biotechnol.* 76(2-3):1 57-63 (2000); Fischer *et al., Biotechnol. Appl. Biochem.* 30 (Pt 2): 117-20 (1999); Pennell *et al., Res. Immunol.* 149(6): 599-603 (1998); Eldin *et al., J. Immunol. Methods.* 201(1): 67-75 (1997);, Frenken *et al., Res. Immunol.* 149(6): 589-99 (1998); Shusta *et al., Nature Biotechnol.* 16(8): 773-7 (1998), the disclosures of which are incorporated herein  
25 by reference in their entirety.

Antibodies, including antibody fragments and derivatives, of the present invention can also be produced in insect cells. *See, e.g.,* Li *et al., Protein Expr. Purif.* 21(1): 121-8 (2001); Ailor *et al., Biotechnol. Bioeng.* 58(2-3): 196-203 (1998); Hsu *et al., Biotechnol. Prog.* 13(1): 96-104 (1997); Edelman *et al., Immunology* 91(1): 13-9 (1997);  
30 and Nesbit *et al., J. Immunol. Methods* 151(1-2): 201-8 (1992), the disclosures of which are incorporated herein by reference in their entirety.

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Antibodies and fragments and derivatives thereof of the present invention can also be produced in plant cells, particularly maize or tobacco, Giddings *et al.*, *Nature Biotechnol.* 18(11): 1151-5 (2000); Gavilondo *et al.*, *Biotechniques* 29(1): 128-38 (2000); Fischer *et al.*, *J. Biol. Regul. Homeost. Agents* 14(2): 83-92 (2000); Fischer *et al.*,  
5 *Biotechnol. Appl. Biochem.* 30 (Pt 2): 113-6 (1999); Fischer *et al.*, *Biol. Chem.* 380(7-8): 825-39 (1999); Russell, *Curr. Top. Microbiol. Immunol.* 240: 119-38 (1999); and Ma *et al.*, *Plant Physiol.* 109(2): 341-6 (1995), the disclosures of which are incorporated herein by reference in their entirety.

Antibodies, including antibody fragments and derivatives, of the present  
10 invention can also be produced in transgenic, non-human, mammalian milk. *See, e.g.* Pollock *et al.*, *J. Immunol Methods.* 231: 147-57 (1999); Young *et al.*, *Res. Immunol.* 149: 609-10 (1998); Limonta *et al.*, *Immunotechnology* 1: 107-13 (1995), the disclosures of which are incorporated herein by reference in their entirety.

Mammalian cells useful for recombinant expression of antibodies, antibody  
15 fragments, and antibody derivatives of the present invention include CHO cells, COS cells, 293 cells, and myeloma cells.

Verma *et al.*, *J. Immunol. Methods* 216(1-2):165-81 (1998), herein incorporated by reference, review and compare bacterial, yeast, insect and mammalian expression systems for expression of antibodies.

20 Antibodies of the present invention can also be prepared by cell free translation, as further described in Merk *et al.*, *J. Biochem.* (Tokyo) 125(2): 328-33 (1999) and Ryabova *et al.*, *Nature Biotechnol.* 15(1): 79-84 (1997), and in the milk of transgenic animals, as further described in Pollock *et al.*, *J. Immunol. Methods* 231(1-2): 147-57 (1999), the disclosures of which are incorporated herein by reference in their entirety.

25 The invention further provides antibody fragments that bind specifically to one or more of the proteins and protein fragments of the present invention, to one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention, or the binding of which can be competitively inhibited by one or more of the proteins and protein fragments of the present invention or one or more of the proteins and  
30 protein fragments encoded by the isolated nucleic acids of the present invention.



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Among such useful fragments are Fab, Fab', Fv, F(ab)'<sub>2</sub>, and single chain Fv (scFv) fragments. Other useful fragments are described in Hudson, *Curr. Opin. Biotechnol.* 9(4): 395-402 (1998).

It is also an aspect of the present invention to provide antibody derivatives that  
5 bind specifically to one or more of the proteins and protein fragments of the present invention, to one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention, or the binding of which can be competitively inhibited by one or more of the proteins and protein fragments of the present invention or  
10 one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention.

Among such useful derivatives are chimeric, primatized, and humanized antibodies; such derivatives are less immunogenic in human beings, and thus more suitable for *in vivo* administration, than are unmodified antibodies from non-human mammalian species. Another useful derivative is PEGylation to increase the serum half  
15 life of the antibodies.

Chimeric antibodies typically include heavy and/or light chain variable regions (including both CDR and framework residues) of immunoglobulins of one species, typically mouse, fused to constant regions of another species, typically human. *See, e.g.*, United States Patent No. 5,807,715; Morrison *et al.*, *Proc. Natl. Acad. Sci USA* 81(21):  
20 6851-5 (1984); Sharon *et al.*, *Nature* 309(5966): 364-7 (1984); Takeda *et al.*, *Nature* 314(6010): 452-4 (1985), the disclosures of which are incorporated herein by reference in their entireties. Primatized and humanized antibodies typically include heavy and/or light chain CDRs from a murine antibody grafted into a non-human primate or human antibody V region framework, usually further comprising a human constant region,  
25 Riechmann *et al.*, *Nature* 332(6162): 323-7 (1988); Co *et al.*, *Nature* 351(6326): 501-2 (1991); United States Patent Nos. 6,054,297; 5,821,337; 5,770,196; 5,766,886; 5,821,123; 5,869,619; 6,180,377; 6,013,256; 5,693,761; and 6,180,370, the disclosures of which are incorporated herein by reference in their entireties.

Other useful antibody derivatives of the invention include heteromeric antibody  
30 complexes and antibody fusions, such as diabodies (bispecific antibodies), single-chain diabodies, and intrabodies.

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It is contemplated that the nucleic acids encoding the antibodies of the present invention can be operably joined to other nucleic acids forming a recombinant vector for cloning or for expression of the antibodies of the invention. The present invention includes any recombinant vector containing the coding sequences, or part thereof, whether for eukaryotic transduction, transfection or gene therapy. Such vectors may be prepared using conventional molecular biology techniques, known to those with skill in the art, and would comprise DNA encoding sequences for the immunoglobulin V-regions including framework and CDRs or parts thereof, and a suitable promoter either with or without a signal sequence for intracellular transport. Such vectors may be transduced or transfected into eukaryotic cells or used for gene therapy (Marasco et al., *Proc. Natl. Acad. Sci. (USA)* 90: 7889-7893 (1993); Duan et al., *Proc. Natl. Acad. Sci. (USA)* 91: 5075-5079 (1994), by conventional techniques, known to those with skill in the art.

The antibodies of the present invention, including fragments and derivatives thereof, can usefully be labeled. It is, therefore, another aspect of the present invention to provide labeled antibodies that bind specifically to one or more of the proteins and protein fragments of the present invention, to one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention, or the binding of which can be competitively inhibited by one or more of the proteins and protein fragments of the present invention or one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention.

The choice of label depends, in part, upon the desired use.

For example, when the antibodies of the present invention are used for immunohistochemical staining of tissue samples, the label is preferably an enzyme that catalyzes production and local deposition of a detectable product.

Enzymes typically conjugated to antibodies to permit their immunohistochemical visualization are well-known, and include alkaline phosphatase,  $\beta$ -galactosidase, glucose oxidase, horseradish peroxidase (HRP), and urease. Typical substrates for production and deposition of visually detectable products include o-nitrophenyl-beta-D-galactopyranoside (ONPG); o-phenylenediamine dihydrochloride (OPD); p-nitrophenyl phosphate (PNPP); p-nitrophenyl-beta-D-galactopyranoside (PNPG); 3',3'-diaminobenzidine (DAB); 3-amino-9-ethylcarbazole (AEC); 4-chloro-1-naphthol (CN); 5-bromo-4-chloro-3-indolyl-phosphate (BCIP); ABTS®; BluoGal; iodonitrotetrazolium

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(INT); nitroblue tetrazolium chloride (NBT); phenazine methosulfate (PMS); phenolphthalein monophosphate (PMP); tetramethyl benzidine (TMB); tetranitroblue tetrazolium (TNBT); X-Gal; X-Gluc; and X-Glucoside.

Other substrates can be used to produce products for local deposition that are luminescent. For example, in the presence of hydrogen peroxide ( $H_2O_2$ ), horseradish peroxidase (HRP) can catalyze the oxidation of cyclic diacylhydrazides, such as luminol. Immediately following the oxidation, the luminol is in an excited state (intermediate reaction product), which decays to the ground state by emitting light. Strong enhancement of the light emission is produced by enhancers, such as phenolic compounds. Advantages include high sensitivity, high resolution, and rapid detection without radioactivity and requiring only small amounts of antibody. See, e.g., Thorpe *et al.*, *Methods Enzymol.* 133: 331-53 (1986); Kricka *et al.*, *J. Immunoassay* 17(1): 67-83 (1996); and Lundqvist *et al.*, *J. Biolumin. Chemilumin.* 10(6): 353-9 (1995), the disclosures of which are incorporated herein by reference in their entireties. Kits for such enhanced chemiluminescent detection (ECL) are available commercially.

The antibodies can also be labeled using colloidal gold.

As another example, when the antibodies of the present invention are used, e.g., for flow cytometric detection, for scanning laser cytometric detection, or for fluorescent immunoassay, they can usefully be labeled with fluorophores.

There are a wide variety of fluorophore labels that can usefully be attached to the antibodies of the present invention.

For flow cytometric applications, both for extracellular detection and for intracellular detection, common useful fluorophores can be fluorescein isothiocyanate (FITC), allophycocyanin (APC), R-phycoerythrin (PE), peridinin chlorophyll protein (PerCP), Texas Red, Cy3, Cy5, fluorescence resonance energy tandem fluorophores such as PerCP-Cy5.5, PE-Cy5, PE-Cy5.5, PE-Cy7, PE-Texas Red, and APC-Cy7.

Other fluorophores include, *inter alia*, Alexa Fluor® 350, Alexa Fluor® 488, Alexa Fluor® 532, Alexa Fluor® 546, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 647 (monoclonal antibody labeling kits available from Molecular Probes, Inc., Eugene, OR, USA), BODIPY dyes, such as BODIPY 493/503, BODIPY FL, BODIPY R6G, BODIPY 530/550, BODIPY TMR, BODIPY 558/568, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589, BODIPY 581/591, BODIPY TR, BODIPY

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630/650, BODIPY 650/665, Cascade Blue, Cascade Yellow, Dansyl, lissamine rhodamine B, Marina Blue, Oregon Green 488, Oregon Green 514, Pacific Blue, rhodamine 6G, rhodamine green, rhodamine red, tetramethylrhodamine, Texas Red (available from Molecular Probes, Inc., Eugene, OR, USA), and Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, all of which are also useful for fluorescently labeling the antibodies of the present invention.

For secondary detection using labeled avidin, streptavidin, captavidin or neutravidin, the antibodies of the present invention can usefully be labeled with biotin.

When the antibodies of the present invention are used, *e.g.*, for Western blotting applications, they can usefully be labeled with radioisotopes, such as  $^{33}\text{P}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^3\text{H}$ , and  $^{125}\text{I}$ .

As another example, when the antibodies of the present invention are used for radioimmunotherapy, the label can usefully be  $^{228}\text{Th}$ ,  $^{227}\text{Ac}$ ,  $^{225}\text{Ac}$ ,  $^{223}\text{Ra}$ ,  $^{213}\text{Bi}$ ,  $^{212}\text{Pb}$ ,  $^{212}\text{Bi}$ ,  $^{211}\text{At}$ ,  $^{203}\text{Pb}$ ,  $^{194}\text{Os}$ ,  $^{188}\text{Re}$ ,  $^{186}\text{Re}$ ,  $^{153}\text{Sm}$ ,  $^{149}\text{Tb}$ ,  $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{111}\text{In}$ ,  $^{105}\text{Rh}$ ,  $^{99\text{m}}\text{Tc}$ ,  $^{97}\text{Ru}$ ,  $^{90}\text{Y}$ ,  $^{90}\text{Sr}$ ,  $^{88}\text{Y}$ ,  $^{72}\text{Se}$ ,  $^{67}\text{Cu}$ , or  $^{47}\text{Sc}$ .

As another example, when the antibodies of the present invention are to be used for *in vivo* diagnostic use, they can be rendered detectable by conjugation to MRI contrast agents, such as gadolinium diethylenetriaminepentaacetic acid (DTPA), Laufer *et al.*, *Radiology* 207(2): 529-38 (1998), or by radioisotopic labeling.

As would be understood, use of the labels described above is not restricted to the application for which they are mentioned.

The antibodies of the present invention, including fragments and derivatives thereof, can also be conjugated to toxins, in order to target the toxin's ablative action to cells that display and/or express the proteins of the present invention. Commonly, the antibody in such immunotoxins is conjugated to *Pseudomonas* exotoxin A, *diphtheria* toxin, *shiga* toxin A, *anthrax* toxin lethal factor, or ricin. See Hall (ed.), Immunotoxin Methods and Protocols (Methods in Molecular Biology, vol. 166), Humana Press (2000); and Frankel *et al.* (eds.), Clinical Applications of Immunotoxins, Springer-Verlag (1998), the disclosures of which are incorporated herein by reference in their entireties.

The antibodies of the present invention can usefully be attached to a substrate, and it is, therefore, another aspect of the invention to provide antibodies that bind specifically to one or more of the proteins and protein fragments of the present invention,

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to one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention, or the binding of which can be competitively inhibited by one or more of the proteins and protein fragments of the present invention or one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention, attached to a substrate.

Substrates can be porous or nonporous, planar or nonplanar.

For example, the antibodies of the present invention can usefully be conjugated to filtration media, such as NHS-activated Sepharose or CNBr-activated Sepharose for purposes of immunoaffinity chromatography.

For example, the antibodies of the present invention can usefully be attached to paramagnetic microspheres, typically by biotin-streptavidin interaction, which microspheres can then be used for isolation of cells that express or display the proteins of the present invention. As another example, the antibodies of the present invention can usefully be attached to the surface of a microtiter plate for ELISA.

As noted above, the antibodies of the present invention can be produced in prokaryotic and eukaryotic cells. It is, therefore, another aspect of the present invention to provide cells that express the antibodies of the present invention, including hybridoma cells, B cells, plasma cells, and host cells recombinantly modified to express the antibodies of the present invention.

In yet a further aspect, the present invention provides aptamers evolved to bind specifically to one or more of the proteins and protein fragments of the present invention, to one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention, or the binding of which can be competitively inhibited by one or more of the proteins and protein fragments of the present invention or one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention.

In sum, one of skill in the art, provided with the teachings of this invention, has available a variety of methods which may be used to alter the biological properties of the antibodies of this invention including methods which would increase or decrease the stability or half-life, immunogenicity, toxicity, affinity or yield of a given antibody molecule, or to alter it in any other way that may render it more suitable for a particular application.

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Transgenic Animals and Cells

In another aspect, the invention provides transgenic cells and non-human organisms comprising nucleic acid molecules of the invention. In a preferred embodiment, the transgenic cells and non-human organisms comprise a nucleic acid molecule encoding a CSP. In a preferred embodiment, the CSP comprises an amino acid sequence selected from SEQ ID NO: 101 through 176, or a fragment, mutein, homologous protein or allelic variant thereof. In another preferred embodiment, the transgenic cells and non-human organism comprise a CSNA of the invention, preferably a CSNA comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 through 100, or a part, substantially similar nucleic acid molecule, allelic variant or hybridizing nucleic acid molecule thereof.

In another embodiment, the transgenic cells and non-human organisms have a targeted disruption or replacement of the endogenous orthologue of the human CSG.

The transgenic cells can be embryonic stem cells or somatic cells. The transgenic non-human organisms can be chimeric, nonchimeric heterozygotes, and nonchimeric homozygotes. Methods of producing transgenic animals are well-known in the art. *See, e.g., Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual*, 2d ed., Cold Spring Harbor Press (1999); Jackson *et al.*, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press (2000); and Pinkert, *Transgenic Animal Technology: A Laboratory Handbook*, Academic Press (1999).

Any technique known in the art may be used to introduce a nucleic acid molecule of the invention into an animal to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection. (*see, e.g., Paterson et al., Appl. Microbiol. Biotechnol.* 40: 691-698 (1994); Carver *et al.*, *Biotechnology* 11: 1263-1270 (1993); Wright *et al.*, *Biotechnology* 9: 830-834 (1991); and U.S. Patent 4,873,191 (1989) retrovirus-mediated gene transfer into germ lines, blastocysts or embryos (*see, e.g., Van der Putten et al., Proc. Natl. Acad. Sci., USA* 82: 6148-6152 (1985)); gene targeting in embryonic stem cells (*see, e.g., Thompson et al., Cell* 56: 313-321 (1989)); electroporation of cells or embryos (*see, e.g., Lo, 1983, Mol. Cell. Biol.* 3: 1803-1814 (1983)); introduction using a gene gun (*see, e.g., Ulmer et al., Science* 259: 1745-49 (1993); introducing nucleic acid constructs into embryonic pluripotent stem

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cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (*see, e.g., Lavitrano et al., Cell* 57: 717-723 (1989)).

Other techniques include, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (*see, e.g.,*  
5 *Campbell et al., Nature* 380: 64-66 (1996); *Wilmot et al., Nature* 385: 810-813 (1997)). The present invention provides for transgenic animals that carry the transgene (*i.e., a*  
nucleic acid molecule of the invention) in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i. e.,* mosaic animals or chimeric animals.

The transgene may be integrated as a single transgene or as multiple copies, such  
10 as in concatamers, *e. g.,* head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by  
following, *e.g.,* the teaching of Lasko *et al. et al., Proc. Natl. Acad. Sci. USA* 89: 6232-6236 (1992). The regulatory sequences required for such a cell-type specific activation  
will depend upon the particular cell type of interest, and will be apparent to those of skill  
15 in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression  
20 of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (RT-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the  
25 transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in  
30 order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to

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both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

5 Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

10 Methods for creating a transgenic animal with a disruption of a targeted gene are also well-known in the art. In general, a vector is designed to comprise some nucleotide sequences homologous to the endogenous targeted gene. The vector is introduced into a cell so that it may integrate, via homologous recombination with chromosomal sequences, into the endogenous gene, thereby disrupting the function of the endogenous  
15 gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type. *See, e.g., Gu et al., Science* 265: 103-106 (1994). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. *See, e.g., Smithies et al., Nature* 317: 230-234 (1985); Thomas *et al., Cell* 51: 503-512 (1987); Thompson *et al., Cell* 5: 313-321 (1989).

20 In one embodiment, a mutant, non-functional nucleic acid molecule of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous nucleic acid sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable  
25 marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications  
30 to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene. *See, e.g., Thomas, supra* and Thompson, *supra*. However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are



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directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (*e.g.*, knockouts) are administered to a patient *in vivo*. Such cells may be obtained from an animal or patient or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (*e.g.*, lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, *e.g.*, by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc.

The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, *e.g.*, in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, *e.g.*, genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. *See, e.g.*, U.S. Patents 5,399,349 and 5,460,959, each of which is incorporated by reference herein in its entirety.

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well-known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

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Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

#### Computer Readable Means

A further aspect of the invention relates to a computer readable means for storing the nucleic acid and amino acid sequences of the instant invention. In a preferred embodiment, the invention provides a computer readable means for storing SEQ ID NO: 1 through 100 and SEQ ID NO: 101 through 176 as described herein, as the complete set of sequences or in any combination. The records of the computer readable means can be accessed for reading and display and for interface with a computer system for the application of programs allowing for the location of data upon a query for data meeting certain criteria, the comparison of sequences, the alignment or ordering of sequences meeting a set of criteria, and the like.

The nucleic acid and amino acid sequences of the invention are particularly useful as components in databases useful for search analyses as well as in sequence analysis algorithms. As used herein, the terms "nucleic acid sequences of the invention" and "amino acid sequences of the invention" mean any detectable chemical or physical characteristic of a polynucleotide or polypeptide of the invention that is or may be reduced to or stored in a computer readable form. These include, without limitation, chromatographic scan data or peak data, photographic data or scan data therefrom, and mass spectrographic data.

This invention provides computer readable media having stored thereon sequences of the invention. A computer readable medium may comprise one or more of the following: a nucleic acid sequence comprising a sequence of a nucleic acid sequence of the invention; an amino acid sequence comprising an amino acid sequence of the invention; a set of nucleic acid sequences wherein at least one of said sequences comprises the sequence of a nucleic acid sequence of the invention; a set of amino acid sequences wherein at least one of said sequences comprises the sequence of an amino acid sequence of the invention; a data set representing a nucleic acid sequence comprising the sequence of one or more nucleic acid sequences of the invention; a data

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set representing a nucleic acid sequence encoding an amino acid sequence comprising the sequence of an amino acid sequence of the invention; a set of nucleic acid sequences wherein at least one of said sequences comprises the sequence of a nucleic acid sequence of the invention; a set of amino acid sequences wherein at least one of said sequences  
5 comprises the sequence of an amino acid sequence of the invention; a data set representing a nucleic acid sequence comprising the sequence of a nucleic acid sequence of the invention; a data set representing a nucleic acid sequence encoding an amino acid sequence comprising the sequence of an amino acid sequence of the invention. The computer readable medium can be any composition of matter used to store information or  
10 data, including, for example, commercially available floppy disks, tapes, hard drives, compact disks, and video disks.

Also provided by the invention are methods for the analysis of character sequences, particularly genetic sequences. Preferred methods of sequence analysis include, for example, methods of sequence homology analysis, such as identity and  
15 similarity analysis, RNA structure analysis, sequence assembly, cladistic analysis, sequence motif analysis, open reading frame determination, nucleic acid base calling, and sequencing chromatogram peak analysis.

A computer-based method is provided for performing nucleic acid sequence identity or similarity identification. This method comprises the steps of providing a  
20 nucleic acid sequence comprising the sequence of a nucleic acid of the invention in a computer readable medium; and comparing said nucleic acid sequence to at least one nucleic acid or amino acid sequence to identify sequence identity or similarity.

A computer-based method is also provided for performing amino acid homology identification, said method comprising the steps of: providing an amino acid sequence  
25 comprising the sequence of an amino acid of the invention in a computer readable medium; and comparing said an amino acid sequence to at least one nucleic acid or an amino acid sequence to identify homology.

A computer-based method is still further provided for assembly of overlapping nucleic acid sequences into a single nucleic acid sequence, said method comprising the  
30 steps of: providing a first nucleic acid sequence comprising the sequence of a nucleic acid of the invention in a computer readable medium; and screening for at least one

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overlapping region between said first nucleic acid sequence and a second nucleic acid sequence.

#### Diagnostic Methods for Colon Cancer

5           The present invention also relates to quantitative and qualitative diagnostic assays and methods for detecting, diagnosing, monitoring, staging and predicting cancers by comparing expression of a CSNA or a CSP in a human patient that has or may have colon cancer, or who is at risk of developing colon cancer, with the expression of a CSNA or a CSP in a normal human control. For purposes of the present invention,  
10   “expression of a CSNA” or “CSNA expression” means the quantity of CSG mRNA that can be measured by any method known in the art or the level of transcription that can be measured by any method known in the art in a cell, tissue, organ or whole patient. Similarly, the term “expression of a CSP” or “CSP expression” means the amount of CSP that can be measured by any method known in the art or the level of translation of a CSG  
15   CSNA that can be measured by any method known in the art.

          The present invention provides methods for diagnosing colon cancer in a patient, in particular squamous cell carcinoma, by analyzing for changes in levels of CSNA or CSP in cells, tissues, organs or bodily fluids compared with levels of CSNA or CSP in  
20   cells, tissues, organs or bodily fluids of preferably the same type from a normal human control, wherein an increase, or decrease in certain cases, in levels of a CSNA or CSP in the patient versus the normal human control is associated with the presence of colon cancer or with a predilection to the disease. In another preferred embodiment, the present invention provides methods for diagnosing colon cancer in a patient by analyzing changes in the structure of the mRNA of a CSG compared to the mRNA from a normal  
25   control. These changes include, without limitation, aberrant splicing, alterations in polyadenylation and/or alterations in 5' nucleotide capping. In yet another preferred embodiment, the present invention provides methods for diagnosing colon cancer in a patient by analyzing changes in a CSP compared to a CSP from a normal control. These changes include, *e.g.*, alterations in glycosylation and/or phosphorylation of the CSP or  
30   subcellular CSP localization.

          In a preferred embodiment, the expression of a CSNA is measured by determining the amount of an mRNA that encodes an amino acid sequence selected from

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SEQ ID NO: 101 through 176, a homolog, an allelic variant, or a fragment thereof. In a more preferred embodiment, the CSNA expression that is measured is the level of expression of a CSNA mRNA selected from SEQ ID NO: 1 through 100, or a hybridizing nucleic acid, homologous nucleic acid or allelic variant thereof, or a part of any of these nucleic acids. CSNA expression may be measured by any method known in the art, such as those described *supra*, including measuring mRNA expression by Northern blot, quantitative or qualitative reverse transcriptase PCR (RT-PCR), microarray, dot or slot blots or *in situ* hybridization. *See, e.g.*, Ausubel (1992), *supra*; Ausubel (1999), *supra*; Sambrook (1989), *supra*; and Sambrook (2001), *supra*. CSNA transcription may be measured by any method known in the art including using a reporter gene hooked up to the promoter of a CSG of interest or doing nuclear run-off assays. Alterations in mRNA structure, *e.g.*, aberrant splicing variants, may be determined by any method known in the art, including, RT-PCR followed by sequencing or restriction analysis. As necessary, CSNA expression may be compared to a known control, such as normal colon nucleic acid, to detect a change in expression.

In another preferred embodiment, the expression of a CSP is measured by determining the level of a CSP having an amino acid sequence selected from the group consisting of SEQ ID NO: 101 through 176, a homolog, an allelic variant, or a fragment thereof. Such levels are preferably determined in at least one of cells, tissues, organs and/or bodily fluids, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for diagnosing over- or underexpression of CSNA or CSP compared to normal control bodily fluids, cells, or tissue samples may be used to diagnose the presence of colon cancer. The expression level of a CSP may be determined by any method known in the art, such as those described *supra*. In a preferred embodiment, the CSP expression level may be determined by radioimmunoassays, competitive-binding assays, ELISA, Western blot, FACS, immunohistochemistry, immunoprecipitation, proteomic approaches: two-dimensional gel electrophoresis (2D electrophoresis) and non-gel-based approaches such as mass spectrometry or protein interaction profiling. *See, e.g.*, Harlow (1999), *supra*; Ausubel (1992), *supra*; and Ausubel (1999), *supra*. Alterations in the CSP structure may be determined by any method known in the art, including, *e.g.*, using antibodies that specifically recognize phosphoserine, phosphothreonine or

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phosphotyrosine residues, two-dimensional polyacrylamide gel electrophoresis (2D PAGE) and/or chemical analysis of amino acid residues of the protein. *Id.*

In a preferred embodiment, a radioimmunoassay (RIA) or an ELISA is used. An antibody specific to a CSP is prepared if one is not already available. In a preferred  
5 embodiment, the antibody is a monoclonal antibody. The anti-CSP antibody is bound to a solid support and any free protein binding sites on the solid support are blocked with a protein such as bovine serum albumin. A sample of interest is incubated with the antibody on the solid support under conditions in which the CSP will bind to the anti-CSP antibody. The sample is removed, the solid support is washed to remove unbound  
10 material, and an anti-CSP antibody that is linked to a detectable reagent (a radioactive substance for RIA and an enzyme for ELISA) is added to the solid support and incubated under conditions in which binding of the CSP to the labeled antibody will occur. After binding, the unbound labeled antibody is removed by washing. For an ELISA, one or more substrates are added to produce a colored reaction product that is based upon the  
15 amount of a CSP in the sample. For an RIA, the solid support is counted for radioactive decay signals by any method known in the art. Quantitative results for both RIA and ELISA typically are obtained by reference to a standard curve.

Other methods to measure CSP levels are known in the art. For instance, a competition assay may be employed wherein an anti-CSP antibody is attached to a solid  
20 support and an allocated amount of a labeled CSP and a sample of interest are incubated with the solid support. The amount of labeled CSP detected which is attached to the solid support can be correlated to the quantity of a CSP in the sample.

Of the proteomic approaches, 2D PAGE is a well-known technique. Isolation of individual proteins from a sample such as serum is accomplished using sequential  
25 separation of proteins by isoelectric point and molecular weight. Typically, polypeptides are first separated by isoelectric point (the first dimension) and then separated by size using an electric current (the second dimension). In general, the second dimension is perpendicular to the first dimension. Because no two proteins with different sequences are identical on the basis of both size and charge, the result of 2D PAGE is a roughly  
30 square gel in which each protein occupies a unique spot. Analysis of the spots with chemical or antibody probes, or subsequent protein microsequencing can reveal the relative abundance of a given protein and the identity of the proteins in the sample.

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Expression levels of a CSNA can be determined by any method known in the art, including PCR and other nucleic acid methods, such as ligase chain reaction (LCR) and nucleic acid sequence based amplification (NASBA), can be used to detect malignant cells for diagnosis and monitoring of various malignancies. For example,

5 reverse-transcriptase PCR (RT-PCR) is a powerful technique which can be used to detect the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species. In RT-PCR, an mRNA species is first reverse transcribed to complementary DNA (cDNA) with use of the enzyme reverse transcriptase; the cDNA is then amplified as in a standard PCR reaction.

10 Hybridization to specific DNA molecules (*e.g.*, oligonucleotides) arrayed on a solid support can be used to both detect the expression of and quantitate the level of expression of one or more CSNAs of interest. In this approach, all or a portion of one or more CSNAs is fixed to a substrate. A sample of interest, which may comprise RNA, *e.g.*, total RNA or polyA-selected mRNA, or a complementary DNA (cDNA) copy of the  
15 RNA is incubated with the solid support under conditions in which hybridization will occur between the DNA on the solid support and the nucleic acid molecules in the sample of interest. Hybridization between the substrate-bound DNA and the nucleic acid molecules in the sample can be detected and quantitated by several means, including, without limitation, radioactive labeling or fluorescent labeling of the nucleic acid  
20 molecule or a secondary molecule designed to detect the hybrid.

The above tests can be carried out on samples derived from a variety of cells, bodily fluids and/or tissue extracts such as homogenates or solubilized tissue obtained from a patient. Tissue extracts are obtained routinely from tissue biopsy and autopsy material. Bodily fluids useful in the present invention include blood, urine, saliva or any  
25 other bodily secretion or derivative thereof. By blood it is meant to include whole blood, plasma, serum or any derivative of blood. In a preferred embodiment, the specimen tested for expression of CSNA or CSP includes, without limitation, colon tissue, fluid obtained by bronchial alveolar lavage (BAL), sputum, colon cells grown in cell culture, blood, serum, lymph node tissue and lymphatic fluid. In another preferred embodiment,  
30 especially when metastasis of a primary colon cancer is known or suspected, specimens include, without limitation, tissues from brain, bone, bone marrow, liver, adrenal glands and colon. In general, the tissues may be sampled by biopsy, including, without

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limitation, needle biopsy, *e.g.*, transthoracic needle aspiration, cervical mediastinoscopy, endoscopic lymph node biopsy, video-assisted thoracoscopy, exploratory thoracotomy, bone marrow biopsy and bone marrow aspiration. *See* Scott, *supra* and Franklin, pp. 529-570, in Kane, *supra*. For early and inexpensive detection, assaying for changes in CSNAs or CSPs in cells in sputum samples may be particularly useful. Methods of obtaining and analyzing sputum samples is disclosed in Franklin, *supra*.

All the methods of the present invention may optionally include determining the expression levels of one or more other cancer markers in addition to determining the expression level of a CSNA or CSP. In many cases, the use of another cancer marker will decrease the likelihood of false positives or false negatives. In one embodiment, the one or more other cancer markers include other CSNA or CSPs as disclosed herein. Other cancer markers useful in the present invention will depend on the cancer being tested and are known to those of skill in the art. In a preferred embodiment, at least one other cancer marker in addition to a particular CSNA or CSP is measured. In a more preferred embodiment, at least two other additional cancer markers are used. In an even more preferred embodiment, at least three, more preferably at least five, even more preferably at least ten additional cancer markers are used.

### *Diagnosing*

In one aspect, the invention provides a method for determining the expression levels and/or structural alterations of one or more CSNAs and/or CSPs in a sample from a patient suspected of having colon cancer. In general, the method comprises the steps of obtaining the sample from the patient, determining the expression level or structural alterations of a CSNA and/or CSP and then ascertaining whether the patient has colon cancer from the expression level of the CSNA or CSP. In general, if high expression relative to a control of a CSNA or CSP is indicative of colon cancer, a diagnostic assay is considered positive if the level of expression of the CSNA or CSP is at least two times higher, and more preferably are at least five times higher, even more preferably at least ten times higher, than in preferably the same cells, tissues or bodily fluid of a normal human control. In contrast, if low expression relative to a control of a CSNA or CSP is indicative of colon cancer, a diagnostic assay is considered positive if the level of expression of the CSNA or CSP is at least two times lower, more preferably are at least five times lower, even more preferably at least ten times lower than in preferably the



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same cells, tissues or bodily fluid of a normal human control. The normal human control may be from a different patient or from uninvolved tissue of the same patient.

The present invention also provides a method of determining whether colon cancer has metastasized in a patient. One may identify whether the colon cancer has metastasized by measuring the expression levels and/or structural alterations of one or more CSNAs and/or CSPs in a variety of tissues. The presence of a CSNA or CSP in a certain tissue at levels higher than that of corresponding noncancerous tissue (*e.g.*, the same tissue from another individual) is indicative of metastasis if high level expression of a CSNA or CSP is associated with colon cancer. Similarly, the presence of a CSNA or CSP in a tissue at levels lower than that of corresponding noncancerous tissue is indicative of metastasis if low level expression of a CSNA or CSP is associated with colon cancer. Further, the presence of a structurally altered CSNA or CSP that is associated with colon cancer is also indicative of metastasis.

In general, if high expression relative to a control of a CSNA or CSP is indicative of metastasis, an assay for metastasis is considered positive if the level of expression of the CSNA or CSP is at least two times higher, and more preferably are at least five times higher, even more preferably at least ten times higher, than in preferably the same cells, tissues or bodily fluid of a normal human control. In contrast, if low expression relative to a control of a CSNA or CSP is indicative of metastasis, an assay for metastasis is considered positive if the level of expression of the CSNA or CSP is at least two times lower, more preferably are at least five times lower, even more preferably at least ten times lower than in preferably the same cells, tissues or bodily fluid of a normal human control.

The CSNA or CSP of this invention may be used as element in an array or a multi-analyte test to recognize expression patterns associated with colon cancers or other colon related disorders. In addition, the sequences of either the nucleic acids or proteins may be used as elements in a computer program for pattern recognition of colon disorders.

### 30 *Staging*

The invention also provides a method of staging colon cancer in a human patient. The method comprises identifying a human patient having colon cancer and analyzing

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cells, tissues or bodily fluids from such human patient for expression levels and/or structural alterations of one or more CSNAs or CSPs. First, one or more tumors from a variety of patients are staged according to procedures well-known in the art, and the expression level of one or more CSNAs or CSPs is determined for each stage to obtain a standard expression level for each CSNA and CSP. Then, the CSNA or CSP expression levels are determined in a biological sample from a patient whose stage of cancer is not known. The CSNA or CSP expression levels from the patient are then compared to the standard expression level. By comparing the expression level of the CSNAs and CSPs from the patient to the standard expression levels, one may determine the stage of the tumor. The same procedure may be followed using structural alterations of a CSNA or CSP to determine the stage of a colon cancer.

### *Monitoring*

Further provided is a method of monitoring colon cancer in a human patient. One may monitor a human patient to determine whether there has been metastasis and, if there has been, when metastasis began to occur. One may also monitor a human patient to determine whether a preneoplastic lesion has become cancerous. One may also monitor a human patient to determine whether a therapy, *e.g.*, chemotherapy, radiotherapy or surgery, has decreased or eliminated the colon cancer. The method comprises identifying a human patient that one wants to monitor for colon cancer, periodically analyzing cells, tissues or bodily fluids from such human patient for expression levels of one or more CSNAs or CSPs, and comparing the CSNA or CSP levels over time to those CSNA or CSP expression levels obtained previously. Patients may also be monitored by measuring one or more structural alterations in a CSNA or CSP that are associated with colon cancer.

If increased expression of a CSNA or CSP is associated with metastasis, treatment failure, or conversion of a preneoplastic lesion to a cancerous lesion, then detecting an increase in the expression level of a CSNA or CSP indicates that the tumor is metastasizing, that treatment has failed or that the lesion is cancerous, respectively. One having ordinary skill in the art would recognize that if this were the case, then a decreased expression level would be indicative of no metastasis, effective therapy or failure to progress to a neoplastic lesion. If decreased expression of a CSNA or CSP is associated with metastasis, treatment failure, or conversion of a preneoplastic lesion to a

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cancerous lesion, then detecting an decrease in the expression level of a CSNA or CSP indicates that the tumor is metastasizing, that treatment has failed or that the lesion is cancerous, respectively. In a preferred embodiment, the levels of CSNAs or CSPs are determined from the same cell type, tissue or bodily fluid as prior patient samples.

- 5 Monitoring a patient for onset of colon cancer metastasis is periodic and preferably is done on a quarterly basis, but may be done more or less frequently.

The methods described herein can further be utilized as prognostic assays to identify subjects having or at risk of developing a disease or disorder associated with increased or decreased expression levels of a CSNA and/or CSP. The present invention  
10 provides a method in which a test sample is obtained from a human patient and one or more CSNAs and/or CSPs are detected. The presence of higher (or lower) CSNA or CSP levels as compared to normal human controls is diagnostic for the human patient being at risk for developing cancer, particularly colon cancer. The effectiveness of therapeutic agents to decrease (or increase) expression or activity of one or more CSNAs and/or  
15 CSPs of the invention can also be monitored by analyzing levels of expression of the CSNAs and/or CSPs in a human patient in clinical trials or in *in vitro* screening assays such as in human cells. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the human patient or cells, as the case may be, to the agent being tested.

20 *Detection of Genetic Lesions or Mutations*

The methods of the present invention can also be used to detect genetic lesions or mutations in a CSG, thereby determining if a human with the genetic lesion is susceptible to developing colon cancer or to determine what genetic lesions are responsible, or are partly responsible, for a person's existing colon cancer. Genetic lesions can be detected,  
25 for example, by ascertaining the existence of a deletion, insertion and/or substitution of one or more nucleotides from the CSGs of this invention, a chromosomal rearrangement of CSG, an aberrant modification of CSG (such as of the methylation pattern of the genomic DNA), or allelic loss of a CSG. Methods to detect such lesions in the CSG of this invention are known to those having ordinary skill in the art following the teachings  
30 of the specification.

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Methods of Detecting Noncancerous Colon Diseases

The invention also provides a method for determining the expression levels and/or structural alterations of one or more CSNAs and/or CSPs in a sample from a patient suspected of having or known to have a noncancerous colon disease. In general, the method comprises the steps of obtaining a sample from the patient, determining the expression level or structural alterations of a CSNA and/or CSP, comparing the expression level or structural alteration of the CSNA or CSP to a normal colon control, and then ascertaining whether the patient has a noncancerous colon disease. In general, if high expression relative to a control of a CSNA or CSP is indicative of a particular noncancerous colon disease, a diagnostic assay is considered positive if the level of expression of the CSNA or CSP is at least two times higher, and more preferably are at least five times higher, even more preferably at least ten times higher, than in preferably the same cells, tissues or bodily fluid of a normal human control. In contrast, if low expression relative to a control of a CSNA or CSP is indicative of a noncancerous colon disease, a diagnostic assay is considered positive if the level of expression of the CSNA or CSP is at least two times lower, more preferably are at least five times lower, even more preferably at least ten times lower than in preferably the same cells, tissues or bodily fluid of a normal human control. The normal human control may be from a different patient or from uninvolved tissue of the same patient.

One having ordinary skill in the art may determine whether a CSNA and/or CSP is associated with a particular noncancerous colon disease by obtaining colon tissue from a patient having a noncancerous colon disease of interest and determining which CSNAs and/or CSPs are expressed in the tissue at either a higher or a lower level than in normal colon tissue. In another embodiment, one may determine whether a CSNA or CSP exhibits structural alterations in a particular noncancerous colon disease state by obtaining colon tissue from a patient having a noncancerous colon disease of interest and determining the structural alterations in one or more CSNAs and/or CSPs relative to normal colon tissue.

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Methods for Identifying Colon Tissue

In another aspect, the invention provides methods for identifying colon tissue. These methods are particularly useful in, *e.g.*, forensic science, colon cell differentiation  
5 and development, and in tissue engineering.

In one embodiment, the invention provides a method for determining whether a sample is colon tissue or has colon tissue-like characteristics. The method comprises the steps of providing a sample suspected of comprising colon tissue or having colon tissue-like characteristics, determining whether the sample expresses one or more CSNAs  
10 and/or CSPs, and, if the sample expresses one or more CSNAs and/or CSPs, concluding that the sample comprises colon tissue. In a preferred embodiment, the CSNA encodes a polypeptide having an amino acid sequence selected from SEQ ID NO: 101 through 176, or a homolog, allelic variant or fragment thereof. In a more preferred embodiment, the CSNA has a nucleotide sequence selected from SEQ ID NO: 1 through 100, or a  
15 hybridizing nucleic acid, an allelic variant or a part thereof. Determining whether a sample expresses a CSNA can be accomplished by any method known in the art. Preferred methods include hybridization to microarrays, Northern blot hybridization, and quantitative or qualitative RT-PCR. In another preferred embodiment, the method can be practiced by determining whether a CSP is expressed. Determining whether a sample  
20 expresses a CSP can be accomplished by any method known in the art. Preferred methods include Western blot, ELISA, RIA and 2D PAGE. In one embodiment, the CSP has an amino acid sequence selected from SEQ ID NO: 101 through 176, or a homolog, allelic variant or fragment thereof. In another preferred embodiment, the expression of at least two CSNAs and/or CSPs is determined. In a more preferred embodiment, the  
25 expression of at least three, more preferably four and even more preferably five CSNAs and/or CSPs are determined.

In one embodiment, the method can be used to determine whether an unknown tissue is colon tissue. This is particularly useful in forensic science, in which small, damaged pieces of tissues that are not identifiable by microscopic or other means are  
30 recovered from a crime or accident scene. In another embodiment, the method can be used to determine whether a tissue is differentiating or developing into colon tissue. This is important in monitoring the effects of the addition of various agents to cell or tissue culture, *e.g.*, in producing new colon tissue by tissue engineering. These agents include,

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e.g., growth and differentiation factors, extracellular matrix proteins and culture medium. Other factors that may be measured for effects on tissue development and differentiation include gene transfer into the cells or tissues, alterations in pH, aqueous:air interface and various other culture conditions.

## 5 Methods for Producing and Modifying Colon Tissue

In another aspect, the invention provides methods for producing engineered colon tissue or cells. In one embodiment, the method comprises the steps of providing cells, introducing a CSNA or a CSG into the cells, and growing the cells under conditions in  
10 which they exhibit one or more properties of colon tissue cells. In a preferred embodiment, the cells are pluripotent. As is well-known in the art, normal colon tissue comprises a large number of different cell types. Thus, in one embodiment, the engineered colon tissue or cells comprises one of these cell types. In another  
15 embodiment, the engineered colon tissue or cells comprises more than one colon cell type. Further, the culture conditions of the cells or tissue may require manipulation in order to achieve full differentiation and development of the colon cell tissue. Methods for manipulating culture conditions are well-known in the art.

Nucleic acid molecules encoding one or more CSPs are introduced into cells, preferably pluripotent cells. In a preferred embodiment, the nucleic acid molecules  
20 encode CSPs having amino acid sequences selected from SEQ ID NO: 101 through 176, or homologous proteins, analogs, allelic variants or fragments thereof. In a more preferred embodiment, the nucleic acid molecules have a nucleotide sequence selected from SEQ ID NO: 1 through 100, or hybridizing nucleic acids, allelic variants or parts thereof. In another highly preferred embodiment, a CSG is introduced into the cells.  
25 Expression vectors and methods of introducing nucleic acid molecules into cells are well-known in the art and are described in detail, *supra*.

Artificial colon tissue may be used to treat patients who have lost some or all of their colon function.

## 30 Pharmaceutical Compositions

In another aspect, the invention provides pharmaceutical compositions comprising the nucleic acid molecules, polypeptides, antibodies, antibody derivatives,

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antibody fragments, agonists, antagonists, and inhibitors of the present invention. In a preferred embodiment, the pharmaceutical composition comprises a CSNA or part thereof. In a more preferred embodiment, the CSNA has a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 through 100, a nucleic acid that hybridizes thereto, an allelic variant thereof, or a nucleic acid that has substantial sequence identity thereto. In another preferred embodiment, the pharmaceutical composition comprises a CSP or fragment thereof. In a more preferred embodiment, the CSP having an amino acid sequence that is selected from the group consisting of SEQ ID NO: 101 through 176, a polypeptide that is homologous thereto, a fusion protein comprising all or a portion of the polypeptide, or an analog or derivative thereof. In another preferred embodiment, the pharmaceutical composition comprises an anti-CSP antibody, preferably an antibody that specifically binds to a CSP having an amino acid that is selected from the group consisting of SEQ ID NO: 101 through 176, or an antibody that binds to a polypeptide that is homologous thereto, a fusion protein comprising all or a portion of the polypeptide, or an analog or derivative thereof.

Such a composition typically contains from about 0.1 to 90% by weight of a therapeutic agent of the invention formulated in and/or with a pharmaceutically acceptable carrier or excipient.

Pharmaceutical formulation is a well-established art, and is further described in Gennaro (ed.), Remington: The Science and Practice of Pharmacy, 20<sup>th</sup> ed., Lippincott, Williams & Wilkins (2000); Ansel *et al.*, Pharmaceutical Dosage Forms and Drug Delivery Systems, 7<sup>th</sup> ed., Lippincott Williams & Wilkins (1999); and Kibbe (ed.), Handbook of Pharmaceutical Excipients American Pharmaceutical Association, 3<sup>rd</sup> ed. (2000), the disclosures of which are incorporated herein by reference in their entireties, and thus need not be described in detail herein.

Briefly, formulation of the pharmaceutical compositions of the present invention will depend upon the route chosen for administration. The pharmaceutical compositions utilized in this invention can be administered by various routes including both enteral and parenteral routes, including oral, intravenous, intramuscular, subcutaneous, inhalation, topical, sublingual, rectal, intra-arterial, intramedullary, intrathecal, intraventricular, transmucosal, transdermal, intranasal, intraperitoneal, intrapulmonary, and intrauterine.

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Oral dosage forms can be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Solid formulations of the compositions for oral administration can contain suitable carriers or excipients, such as carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, or microcrystalline cellulose; gums including arabic and tragacanth; proteins such as gelatin and collagen; inorganics, such as kaolin, calcium carbonate, dicalcium phosphate, sodium chloride; and other agents such as acacia and alginic acid.

Agents that facilitate disintegration and/or solubilization can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate, microcrystalline cellulose, corn starch, sodium starch glycolate, and alginic acid.

Tablet binders that can be used include acacia, methylcellulose, sodium carboxymethylcellulose, polyvinylpyrrolidone (Povidone™), hydroxypropyl methylcellulose, sucrose, starch and ethylcellulose.

Lubricants that can be used include magnesium stearates, stearic acid, silicone fluid, talc, waxes, oils, and colloidal silica.

Fillers, agents that facilitate disintegration and/or solubilization, tablet binders and lubricants, including the aforementioned, can be used singly or in combination.

Solid oral dosage forms need not be uniform throughout. For example, dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which can also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures.

Oral dosage forms of the present invention include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or



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suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Additionally, dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

5        Liquid formulations of the pharmaceutical compositions for oral (enteral) administration are prepared in water or other aqueous vehicles and can contain various suspending agents such as methylcellulose, alginates, tragacanth, pectin, kelgin, carrageenan, acacia, polyvinylpyrrolidone, and polyvinyl alcohol. The liquid formulations can also include solutions, emulsions, syrups and elixirs containing,  
10 together with the active compound(s), wetting agents, sweeteners, and coloring and flavoring agents.

The pharmaceutical compositions of the present invention can also be formulated for parenteral administration. Formulations for parenteral administration can be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions.

15        For intravenous injection, water soluble versions of the compounds of the present invention are formulated in, or if provided as a lyophilate, mixed with, a physiologically acceptable fluid vehicle, such as 5% dextrose ("D5"), physiologically buffered saline, 0.9% saline, Hanks' solution, or Ringer's solution. Intravenous formulations may include carriers, excipients or stabilizers including, without limitation, calcium, human serum  
20 albumin, citrate, acetate, calcium chloride, carbonate, and other salts.

Intramuscular preparations, *e.g.* a sterile formulation of a suitable soluble salt form of the compounds of the present invention, can be dissolved and administered in a pharmaceutical excipient such as Water-for-Injection, 0.9% saline, or 5% glucose solution. Alternatively, a suitable insoluble form of the compound can be prepared and  
25 administered as a suspension in an aqueous base or a pharmaceutically acceptable oil base, such as an ester of a long chain fatty acid (*e.g.*, ethyl oleate), fatty oils such as sesame oil, triglycerides, or liposomes.

Parenteral formulations of the compositions can contain various carriers such as vegetable oils, dimethylacetamide, dimethylformamide, ethyl lactate, ethyl carbonate,  
30 isopropyl myristate, ethanol, polyols (glycerol, propylene glycol, liquid polyethylene glycol, and the like).

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Aqueous injection suspensions can also contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Non-lipid polycationic amino polymers can also be used for delivery. Optionally, the suspension can also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical compositions of the present invention can also be formulated to permit injectable, long-term, deposition. Injectable depot forms may be made by forming microencapsulated matrices of the compound in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in microemulsions that are compatible with body tissues.

The pharmaceutical compositions of the present invention can be administered topically.

For topical use the compounds of the present invention can also be prepared in suitable forms to be applied to the skin, or mucus membranes of the nose and throat, and can take the form of lotions, creams, ointments, liquid sprays or inhalants, drops, tinctures, lozenges, or throat paints. Such topical formulations further can include chemical compounds such as dimethylsulfoxide (DMSO) to facilitate surface penetration of the active ingredient. In other transdermal formulations, typically in patch-delivered formulations, the pharmaceutically active compound is formulated with one or more skin penetrants, such as 2-N-methyl-pyrrolidone (NMP) or Azone. A topical semi-solid ointment formulation typically contains a concentration of the active ingredient from about 1 to 20%, *e.g.*, 5 to 10%, in a carrier such as a pharmaceutical cream base.

For application to the eyes or ears, the compounds of the present invention can be presented in liquid or semi-liquid form formulated in hydrophobic or hydrophilic bases as ointments, creams, lotions, paints or powders.

For rectal administration the compounds of the present invention can be administered in the form of suppositories admixed with conventional carriers such as cocoa butter, wax or other glyceride.

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Inhalation formulations can also readily be formulated. For inhalation, various powder and liquid formulations can be prepared. For aerosol preparations, a sterile formulation of the compound or salt form of the compound may be used in inhalers, such as metered dose inhalers, and nebulizers. Aerosolized forms may be especially useful for treating respiratory disorders.

Alternatively, the compounds of the present invention can be in powder form for reconstitution in the appropriate pharmaceutically acceptable carrier at the time of delivery.

The pharmaceutically active compound in the pharmaceutical compositions of the present invention can be provided as the salt of a variety of acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acid. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms.

After pharmaceutical compositions have been prepared, they are packaged in an appropriate container and labeled for treatment of an indicated condition.

The active compound will be present in an amount effective to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

A "therapeutically effective dose" refers to that amount of active ingredient, for example CSP polypeptide, fusion protein, or fragments thereof, antibodies specific for CSP, agonists, antagonists or inhibitors of CSP, which ameliorates the signs or symptoms of the disease or prevents progression thereof; as would be understood in the medical arts, cure, although desired, is not required.

The therapeutically effective dose of the pharmaceutical agents of the present invention can be estimated initially by *in vitro* tests, such as cell culture assays, followed by assay in model animals, usually mice, rats, rabbits, dogs, or pigs. The animal model can also be used to determine an initial preferred concentration range and route of administration.

For example, the ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population) can be determined in one or more cell culture of animal model systems. The dose ratio of toxic to therapeutic

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effects is the therapeutic index, which can be expressed as LD50/ED50. Pharmaceutical compositions that exhibit large therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies are used in formulating an initial dosage range for human use, and preferably provide a range of circulating concentrations that includes the ED50 with little or no toxicity. After administration, or between successive administrations, the circulating concentration of active agent varies within this range depending upon pharmacokinetic factors well-known in the art, such as the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors specific to the subject requiring treatment. Factors that can be taken into account by the practitioner include the severity of the disease state, general health of the subject, age, weight, gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Where the therapeutic agent is a protein or antibody of the present invention, the therapeutic protein or antibody agent typically is administered at a daily dosage of 0.01 mg to 30 mg/kg of body weight of the patient (*e.g.*, 1 mg/kg to 5 mg/kg). The pharmaceutical formulation can be administered in multiple doses per day, if desired, to achieve the total desired daily dose.

Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

Conventional methods, known to those of ordinary skill in the art of medicine, can be used to administer the pharmaceutical formulation(s) of the present invention to the patient. The pharmaceutical compositions of the present invention can be administered alone, or in combination with other therapeutic agents or interventions.

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Therapeutic Methods

The present invention further provides methods of treating subjects having defects in a gene of the invention, *e.g.*, in expression, activity, distribution, localization, and/or solubility, which can manifest as a disorder of colon function. As used herein, “treating” includes all medically-acceptable types of therapeutic intervention, including palliation and prophylaxis (prevention) of disease. The term “treating” encompasses any improvement of a disease, including minor improvements. These methods are discussed below.

10 *Gene Therapy and Vaccines*

The isolated nucleic acids of the present invention can also be used to drive *in vivo* expression of the polypeptides of the present invention. *In vivo* expression can be driven from a vector, typically a viral vector, often a vector based upon a replication incompetent retrovirus, an adenovirus, or an adeno-associated virus (AAV), for purpose of gene therapy. *In vivo* expression can also be driven from signals endogenous to the nucleic acid or from a vector, often a plasmid vector, such as pVAX1 (Invitrogen, Carlsbad, CA, USA), for purpose of “naked” nucleic acid vaccination, as further described in U.S. Patents 5,589,466; 5,679,647; 5,804,566; 5,830,877; 5,843,913; 5,880,104; 5,958,891; 5,985,847; 6,017,897; 6,110,898; and 6,204,250, the disclosures of which are incorporated herein by reference in their entireties. For cancer therapy, it is preferred that the vector also be tumor-selective. *See, e.g.*, Doronin *et al.*, *J. Virol.* 75: 3314-24 (2001).

In another embodiment of the therapeutic methods of the present invention, a therapeutically effective amount of a pharmaceutical composition comprising a nucleic acid of the present invention is administered. The nucleic acid can be delivered in a vector that drives expression of a CSP, fusion protein, or fragment thereof, or without such vector. Nucleic acid compositions that can drive expression of a CSP are administered, for example, to complement a deficiency in the native CSP, or as DNA vaccines. Expression vectors derived from virus, replication deficient retroviruses, adenovirus, adeno-associated (AAV) virus, herpes virus, or vaccinia virus can be used as can plasmids. *See, e.g.*, Cid-Arregui, *supra*. In a preferred embodiment, the nucleic acid

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molecule encodes a CSP having the amino acid sequence of SEQ ID NO: 101 through 176, or a fragment, fusion protein, allelic variant or homolog thereof.

In still other therapeutic methods of the present invention, pharmaceutical compositions comprising host cells that express a CSP, fusions, or fragments thereof can be administered. In such cases, the cells are typically autologous, so as to circumvent xenogeneic or allotypic rejection, and are administered to complement defects in CSP production or activity. In a preferred embodiment, the nucleic acid molecules in the cells encode a CSP having the amino acid sequence of SEQ ID NO: 101 through 176, or a fragment, fusion protein, allelic variant or homolog thereof.

#### 10 *Antisense Administration*

Antisense nucleic acid compositions, or vectors that drive expression of a CSG antisense nucleic acid, are administered to downregulate transcription and/or translation of a CSG in circumstances in which excessive production, or production of aberrant protein, is the pathophysiologic basis of disease.

15 Antisense compositions useful in therapy can have a sequence that is complementary to coding or to noncoding regions of a CSG. For example, oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred.

Catalytic antisense compositions, such as ribozymes, that are capable of sequence-specific hybridization to CSG transcripts, are also useful in therapy. *See, e.g.*, Phylactou, *Adv. Drug Deliv. Rev.* 44(2-3): 97-108 (2000); Phylactou *et al.*, *Hum. Mol. Genet.* 7(10): 1649-53 (1998); Rossi, *Ciba Found. Symp.* 209: 195-204 (1997); and Sigurdsson *et al.*, *Trends Biotechnol.* 13(8): 286-9 (1995), the disclosures of which are incorporated herein by reference in their entirety.

25 Other nucleic acids useful in the therapeutic methods of the present invention are those that are capable of triplex helix formation in or near the CSG genomic locus. Such triplexing oligonucleotides are able to inhibit transcription. *See, e.g.*, Intody *et al.*, *Nucleic Acids Res.* 28(21): 4283-90 (2000); McGuffie *et al.*, *Cancer Res.* 60(14): 3790-9 (2000), the disclosures of which are incorporated herein by reference. Pharmaceutical compositions comprising such triplex forming oligos (TFOs) are administered in circumstances in which excessive production, or production of aberrant protein, is a pathophysiologic basis of disease.

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In a preferred embodiment, the antisense molecule is derived from a nucleic acid molecule encoding a CSP, preferably a CSP comprising an amino acid sequence of SEQ ID NO: 101 through 176, or a fragment, allelic variant or homolog thereof. In a more preferred embodiment, the antisense molecule is derived from a nucleic acid molecule having a nucleotide sequence of SEQ ID NO: 1 through 100, or a part, allelic variant, substantially similar or hybridizing nucleic acid thereof.

#### *Polypeptide Administration*

In one embodiment of the therapeutic methods of the present invention, a therapeutically effective amount of a pharmaceutical composition comprising a CSP, a fusion protein, fragment, analog or derivative thereof is administered to a subject with a clinically-significant CSP defect.

Protein compositions are administered, for example, to complement a deficiency in native CSP. In other embodiments, protein compositions are administered as a vaccine to elicit a humoral and/or cellular immune response to CSP. The immune response can be used to modulate activity of CSP or, depending on the immunogen, to immunize against aberrant or aberrantly expressed forms, such as mutant or inappropriately expressed isoforms. In yet other embodiments, protein fusions having a toxic moiety are administered to ablate cells that aberrantly accumulate CSP.

In a preferred embodiment, the polypeptide is a CSP comprising an amino acid sequence of SEQ ID NO: 101 through 176, or a fusion protein, allelic variant, homolog, analog or derivative thereof. In a more preferred embodiment, the polypeptide is encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID NO: 1 through 100, or a part, allelic variant, substantially similar or hybridizing nucleic acid thereof.

#### *Antibody, Agonist and Antagonist Administration*

In another embodiment of the therapeutic methods of the present invention, a therapeutically effective amount of a pharmaceutical composition comprising an antibody (including fragment or derivative thereof) of the present invention is administered. As is well-known, antibody compositions are administered, for example, to antagonize activity of CSP, or to target therapeutic agents to sites of CSP presence and/or accumulation. In a preferred embodiment, the antibody specifically binds to a

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CSP comprising an amino acid sequence of SEQ ID NO: 101 through 176, or a fusion protein, allelic variant, homolog, analog or derivative thereof. In a more preferred embodiment, the antibody specifically binds to a CSP encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID NO: 1 through 100, or a part, allelic  
5 variant, substantially similar or hybridizing nucleic acid thereof.

The present invention also provides methods for identifying modulators which bind to a CSP or have a modulatory effect on the expression or activity of a CSP. Modulators which decrease the expression or activity of CSP (antagonists) are believed to be useful in treating colon cancer. Such screening assays are known to those of skill in  
10 the art and include, without limitation, cell-based assays and cell-free assays. Small molecules predicted via computer imaging to specifically bind to regions of a CSP can also be designed, synthesized and tested for use in the imaging and treatment of colon cancer. Further, libraries of molecules can be screened for potential anticancer agents by assessing the ability of the molecule to bind to the CSPs identified herein. Molecules  
15 identified in the library as being capable of binding to a CSP are key candidates for further evaluation for use in the treatment of colon cancer. In a preferred embodiment, these molecules will downregulate expression and/or activity of a CSP in cells.

In another embodiment of the therapeutic methods of the present invention, a pharmaceutical composition comprising a non-antibody antagonist of CSP is  
20 administered. Antagonists of CSP can be produced using methods generally known in the art. In particular, purified CSP can be used to screen libraries of pharmaceutical agents, often combinatorial libraries of small molecules, to identify those that specifically bind and antagonize at least one activity of a CSP.

In other embodiments a pharmaceutical composition comprising an agonist of a  
25 CSP is administered. Agonists can be identified using methods analogous to those used to identify antagonists.

In a preferred embodiment, the antagonist or agonist specifically binds to and antagonizes or agonizes, respectively, a CSP comprising an amino acid sequence of SEQ ID NO: 101 through 176, or a fusion protein, allelic variant, homolog, analog or  
30 derivative thereof. In a more preferred embodiment, the antagonist or agonist specifically binds to and antagonizes or agonizes, respectively, a CSP encoded by a



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nucleic acid molecule having a nucleotide sequence of SEQ ID NO: 1 through 100, or a part, allelic variant, substantially similar or hybridizing nucleic acid thereof.

#### *Targeting Colon Tissue*

The invention also provides a method in which a polypeptide of the invention, or an antibody thereto, is linked to a therapeutic agent such that it can be delivered to the colon or to specific cells in the colon. In a preferred embodiment, an anti-CSP antibody is linked to a therapeutic agent and is administered to a patient in need of such therapeutic agent. The therapeutic agent may be a toxin, if colon tissue needs to be selectively destroyed. This would be useful for targeting and killing colon cancer cells. In another embodiment, the therapeutic agent may be a growth or differentiation factor, which would be useful for promoting colon cell function.

In another embodiment, an anti-CSP antibody may be linked to an imaging agent that can be detected using, *e.g.*, magnetic resonance imaging, CT or PET. This would be useful for determining and monitoring colon function, identifying colon cancer tumors, and identifying noncancerous colon diseases.

### **EXAMPLES**

#### **Example 1: Gene Expression analysis**

CSGs were identified by a systematic analysis of gene expression data in the LIFESEQ® Gold database available from Incyte Genomics Inc (Palo Alto, CA) using the data mining software package CLASP™ (Candidate Lead Automatic Search Program). CLASP™ is a set of algorithms that interrogate Incyte's database to identify genes that are both specific to particular tissue types as well as differentially expressed in tissues from patients with cancer. LifeSeq® Gold contains information about which genes are expressed in various tissues in the body and about the dynamics of expression in both normal and diseased states. CLASP™ first sorts the LifeSeq® Gold database into defined tissue types, such as colon, ovary and prostate. CLASP™ categorizes each tissue sample by disease state. Disease states include "healthy," "cancer," "associated with cancer," "other disease" and "other." Categorizing the disease states improves our ability to identify tissue and cancer-specific molecular targets. CLASP™ then performs a simultaneous parallel search for genes that are expressed both (1) selectively in the defined tissue type compared to other tissue types and (2) differentially in the "cancer"

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disease state compared to the other disease states affecting the same, or different, tissues. This sorting is accomplished by using mathematical and statistical filters that specify the minimum change in expression levels and the minimum frequency that the differential expression pattern must be observed across the tissue samples for the gene to be  
5 considered statistically significant. The CLASP™ algorithm quantifies the relative abundance of a particular gene in each tissue type and in each disease state.

To find the CSGs of this invention, the following specific CLASP™ profiles were utilized: tissue-specific expression (CLASP 1), detectable expression only in cancer tissue (CLASP 2), highest differential expression for a given cancer (CLASP 4);  
10 differential expression in cancer tissue (CLASP 5), and. cDNA libraries were divided into 60 unique tissue types (early versions of LifeSeq® had 48 tissue types). Genes or ESTs were grouped into “gene bins,” where each bin is a cluster of sequences grouped together where they share a common contig. The expression level for each gene bin was calculated for each tissue type. Differential expression significance was calculated with  
15 rigorous statistical significant testing taking into account variations in sample size and relative gene abundance in different libraries and within each library (for the equations used to determine statistically significant expression see Audic and Claverie “The significance of digital gene expression profiles,” Genome Res 7(10): 986-995 (1997), including Equation 1 on page 987 and Equation 2 on page 988, the contents of which are  
20 incorporated by reference). Differentially expressed tissue-specific genes were selected based on the percentage abundance level in the targeted tissue versus all the other tissues (tissue-specificity). The expression levels for each gene in libraries of normal tissues or non-tumor tissues from cancer patients were compared with the expression levels in tissue libraries associated with tumor or disease (cancer-specificity). The results were  
25 analyzed for statistical significance.

The selection of the target genes meeting the rigorous CLASP™ profile criteria were as follows:

- (a) CLASP 1: tissue-specific expression: To qualify as a CLASP 1 candidate, a  
30 gene must exhibit statistically significant expression in the tissue of interest compared to all other tissues. Only if the gene exhibits such differential expression with a 90% of confidence level is it selected as a CLASP 1 candidate.

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- (b) CLASP 2: detectable expression only in cancer tissue: To qualify as a CLASP 2 candidate, a gene must exhibit detectable expression in tumor tissues and undetectable expression in libraries from normal individuals and libraries from normal tissue obtained from diseased patients. In addition, such a gene must also exhibit further specificity for the tumor tissues of interest.
- (c) CLASP 5: differential expression in cancer tissue: To qualify as a CLASP 5 candidate, a gene must be differentially expressed in tumor libraries in the tissue of interest compared to normal libraries for all tissues. Only if the gene exhibits such differential expression with a 90% of confidence level is it selected as a CLASP 5 candidate.

The CLASP™ scores for SEQ ID NO: 1-100 are listed below:

	SEQ ID NO: 1	DEX0255_1	CLASP2
	SEQ ID NO: 2	DEX0255_2	CLASP2
15	SEQ ID NO: 3	DEX0255_3	CLASP2
	SEQ ID NO: 4	DEX0255_4	CLASP2 CLASP1
	SEQ ID NO: 5	DEX0255_5	CLASP2
	SEQ ID NO: 6	DEX0255_6	CLASP2
	SEQ ID NO: 7	DEX0255_7	CLASP2
20	SEQ ID NO: 8	DEX0255_8	CLASP2
	SEQ ID NO: 9	DEX0255_9	CLASP2
	SEQ ID NO: 10	DEX0255_10	CLASP2
	SEQ ID NO: 11	DEX0255_11	CLASP2
	SEQ ID NO: 12	DEX0255_12	CLASP2
25	SEQ ID NO: 13	DEX0255_13	CLASP2
	SEQ ID NO: 14	DEX0255_14	CLASP5 CLASP1
	SEQ ID NO: 15	DEX0255_15	CLASP5 CLASP1
	SEQ ID NO: 16	DEX0255_16	CLASP2
	SEQ ID NO: 17	DEX0255_17	CLASP2
30	SEQ ID NO: 18	DEX0255_18	CLASP5 CLASP1
	SEQ ID NO: 19	DEX0255_19	CLASP5 CLASP1
	SEQ ID NO: 20	DEX0255_20	CLASP5 CLASP1
	SEQ ID NO: 21	DEX0255_21	CLASP5 CLASP1
	SEQ ID NO: 22	DEX0255_22	CLASP2
35	SEQ ID NO: 23	DEX0255_23	CLASP2
	SEQ ID NO: 24	DEX0255_24	CLASP2 CLASP1
	SEQ ID NO: 25	DEX0255_25	CLASP2 CLASP1
	SEQ ID NO: 26	DEX0255_26	CLASP2
	SEQ ID NO: 27	DEX0255_27	CLASP2
40	SEQ ID NO: 28	DEX0255_28	CLASP2 CLASP1
	SEQ ID NO: 29	DEX0255_29	CLASP2 CLASP1
	SEQ ID NO: 30	DEX0255_30	CLASP5 CLASP1

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	SEQ ID NO: 31	DEX0255_31 CLASP2
	SEQ ID NO: 32	DEX0255_32 CLASP5 CLASP1
	SEQ ID NO: 33	DEX0255_33 CLASP5 CLASP1
	SEQ ID NO: 34	DEX0255_34 CLASP2
5	SEQ ID NO: 35	DEX0255_35 CLASP2
	SEQ ID NO: 36	DEX0255_36 CLASP2
	SEQ ID NO: 37	DEX0255_37 CLASP2
	SEQ ID NO: 39	DEX0255_39 CLASP2
	SEQ ID NO: 40	DEX0255_40 CLASP2
10	SEQ ID NO: 41	DEX0255_41 CLASP2
	SEQ ID NO: 42	DEX0255_42 CLASP2
	SEQ ID NO: 43	DEX0255_43 CLASP2
	SEQ ID NO: 44	DEX0255_44 CLASP2
	SEQ ID NO: 45	DEX0255_45 CLASP2
15	SEQ ID NO: 46	DEX0255_46 CLASP2 CLASP1
	SEQ ID NO: 47	DEX0255_47 CLASP2
	SEQ ID NO: 48	DEX0255_48 CLASP2
	SEQ ID NO: 49	DEX0255_49 CLASP2
	SEQ ID NO: 50	DEX0255_50 CLASP2
20	SEQ ID NO: 51	DEX0255_51 CLASP2
	SEQ ID NO: 52	DEX0255_52 CLASP2
	SEQ ID NO: 53	DEX0255_53 CLASP2
	SEQ ID NO: 54	DEX0255_54 CLASP2
	SEQ ID NO: 55	DEX0255_55 CLASP2
25	SEQ ID NO: 56	DEX0255_56 CLASP2
	SEQ ID NO: 57	DEX0255_57 CLASP2 CLASP1
	SEQ ID NO: 58	DEX0255_58 CLASP2 CLASP1
	SEQ ID NO: 59	DEX0255_59 CLASP2
	SEQ ID NO: 60	DEX0255_60 CLASP2
30	SEQ ID NO: 61	DEX0255_61 CLASP2
	SEQ ID NO: 62	DEX0255_62 CLASP2
	SEQ ID NO: 63	DEX0255_63 CLASP2
	SEQ ID NO: 64	DEX0255_64 CLASP5 CLASP1
	SEQ ID NO: 65	DEX0255_65 CLASP5 CLASP1
35	SEQ ID NO: 66	DEX0255_66 CLASP5 CLASP1
	SEQ ID NO: 67	DEX0255_67 CLASP5 CLASP1
	SEQ ID NO: 68	DEX0255_68 CLASP2
	SEQ ID NO: 69	DEX0255_69 CLASP2
	SEQ ID NO: 70	DEX0255_70 CLASP2
40	SEQ ID NO: 71	DEX0255_71 CLASP5 CLASP1
	SEQ ID NO: 72	DEX0255_72 CLASP2
	SEQ ID NO: 73	DEX0255_73 CLASP2
	SEQ ID NO: 74	DEX0255_74 CLASP2
	SEQ ID NO: 75	DEX0255_75 CLASP2
45	SEQ ID NO: 76	DEX0255_76 CLASP2
	SEQ ID NO: 77	DEX0255_77 CLASP2
	SEQ ID NO: 78	DEX0255_78 CLASP2
	SEQ ID NO: 79	DEX0255_79 CLASP2

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	SEQ ID NO: 80	DEX0255_80 CLASP2
	SEQ ID NO: 81	DEX0255_81 CLASP2
	SEQ ID NO: 83	DEX0255_83 CLASP2
	SEQ ID NO: 84	DEX0255_84 CLASP2
5	SEQ ID NO: 85	DEX0255_85 CLASP2
	SEQ ID NO: 87	DEX0255_87 CLASP2 CLASP1
	SEQ ID NO: 88	DEX0255_88 CLASP2 CLASP1
	SEQ ID NO: 91	DEX0255_91 CLASP2
	SEQ ID NO: 92	DEX0255_92 CLASP2
10	SEQ ID NO: 93	DEX0255_93 CLASP2
	SEQ ID NO: 94	DEX0255_94 CLASP2
	SEQ ID NO: 95	DEX0255_95 CLASP2 CLASP1
	SEQ ID NO: 96	DEX0255_96 CLASP5 CLASP1
	SEQ ID NO: 97	DEX0255_97 CLASP5 CLASP1
15	SEQ ID NO: 98	DEX0255_98 CLASP5 CLASP1
	SEQ ID NO: 99	DEX0255_99 CLASP5 CLASP1
	SEQ ID NO: 100	DEX0255_100 CLASP5 CLASP1

### Example 2: Relative Quantitation of Gene Expression

20 Real-Time quantitative PCR with fluorescent Taqman probes is a quantitation detection system utilizing the 5'-3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected

25 by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA). Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ATPase, or 18S ribosomal RNA (rRNA) is used as this

30 endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample were used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" can be obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

35 The tissue distribution and the level of the target gene are evaluated for every sample in normal and cancer tissues. Total RNA is extracted from normal tissues, cancer tissues, and from cancers and the corresponding matched adjacent tissues. Subsequently, first strand cDNA is prepared with reverse transcriptase and the polymerase chain

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reaction is done using primers and Taqman probes specific to each target gene. The results are analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the target gene in a particular tissue compared to the calibrator tissue.

5           One of ordinary skill can design appropriate primers. The relative levels of expression of the CSNA versus normal tissues and other cancer tissues can then be determined. All the values are compared to normal thymus (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

10           The relative levels of expression of the CSNA in pairs of matching samples and 1 cancer and 1 normal/normal adjacent of tissue may also be determined. All the values are compared to normal thymus (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

15           In the analysis of matching samples, the CSNAs that show a high degree of tissue specificity for the tissue of interest. These results confirm the tissue specificity results obtained with normal pooled samples.

          Further, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual are compared. This comparison provides an  
20   indication of specificity for the cancer stage (*e.g.* higher levels of mRNA expression in the cancer sample compared to the normal adjacent).

          Altogether, the high level of tissue specificity, plus the mRNA overexpression in matching samples tested are indicative of SEQ ID NO: 1 through 100 being a diagnostic marker for cancer.

### 25   **Example 3: Protein Expression**

          The CSNA is amplified by polymerase chain reaction (PCR) and the amplified DNA fragment encoding the CSNA is subcloned in pET-21d for expression in *E. coli*. In addition to the CSNA coding sequence, codons for two amino acids, Met-Ala, flanking the NH<sub>2</sub>-terminus of the coding sequence of CSNA, and six histidines, flanking the  
30   COOH-terminus of the coding sequence of CSNA, are incorporated to serve as initiating Met/restriction site and purification tag, respectively.

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An over-expressed protein band of the appropriate molecular weight may be observed on a Coomassie blue stained polyacrylamide gel. This protein band is confirmed by Western blot analysis using monoclonal antibody against 6X Histidine tag.

Large-scale purification of CSP was achieved using cell paste generated from 6-liter bacterial cultures, and purified using immobilized metal affinity chromatography (IMAC). Soluble fractions that had been separated from total cell lysate were incubated with a nickle chelating resin. The column was packed and washed with five column volumes of wash buffer. CSP was eluted stepwise with various concentration imidazole buffers.

#### 10 Example 4: Protein Fusions

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector. For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 2, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced. If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. *See, e. g.,* WO 96/34891.

#### 25 Example 5: Production of an Antibody from a Polypeptide

In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100, µg/ml of streptomycin. The

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splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP20), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT  
 5 medium, and then cloned by limiting dilution as described by Wands *et al.*, *Gastroenterology* 80: 225-232 (1981).

The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide. Alternatively, additional antibodies capable of binding to the polypeptide can be  
 10 produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the  
 15 hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies. Using the Jameson-Wolf methods the following epitopes were predicted. (Jameson and  
 20 Wolf, CABIOS, 4(1), 181-186, 1988, the contents of which are incorporated by reference).

	DEX0255_105	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
	38-48	1.01	11
25	DEX0255_108	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
	35-46	1.06	12
	3-24	1.02	22
	DEX0255_110	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
30	7-23	1.17	17
	DEX0255_111	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
	32-43	0.95	12
35	DEX0255_115	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
	114-124	1.04	11
	61-112	1.03	52



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	DEX0255_116	Antigenicity Index(Jameson-Wolf)
	positions	AI avg length
	27-41	1.10 15
	DEX0255_120	Antigenicity Index(Jameson-Wolf)
5	positions	AI avg length
	12-78	1.02 67
	DEX0255_123	Antigenicity Index(Jameson-Wolf)
	positions	AI avg length
	46-59	1.09 14
10	DEX0255_126	Antigenicity Index(Jameson-Wolf)
	positions	AI avg length
	3-23	1.15 21
	DEX0255_127	Antigenicity Index(Jameson-Wolf)
	positions	AI avg length
15	35-49	1.10 15
	DEX0255_136	Antigenicity Index(Jameson-Wolf)
	positions	AI avg length
	4-31	0.98 28
	DEX0255_138	Antigenicity Index(Jameson-Wolf)
20	positions	AI avg length
	28-54	0.92 27
	DEX0255_139	Antigenicity Index(Jameson-Wolf)
	positions	AI avg length
	18-44	1.06 27
25	DEX0255_140	Antigenicity Index(Jameson-Wolf)
	positions	AI avg length
	37-61	1.03 25
	DEX0255_141	Antigenicity Index(Jameson-Wolf)
	positions	AI avg length
30	240-253	1.22 14
	780-793	1.22 14
	813-822	1.21 10
	542-551	1.21 10
	273-282	1.17 10
35	335-344	1.12 10
	509-523	1.12 15
	110-161	1.10 52
	204-222	1.10 19
	635-721	1.09 87
40	366-450	1.09 85
	723-740	1.08 18
	452-469	1.07 18
	285-303	1.07 19
	834-846	1.04 13
45	43-90	1.02 48
	92-105	0.97 14
	744-762	0.97 19
	314-327	0.96 14

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	473-491	0.94	19
	583-596	0.93	14
	554-572	0.93	19
	DEX0255_145	Antigenicity Index(Jameson-Wolf)	
5	positions	AI	avg length
	11-24	1.17	14
	DEX0255_146	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
10	16-35	0.97	20
	DEX0255_147	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
	18-32	1.08	15
	DEX0255_152	Antigenicity Index(Jameson-Wolf)	
15	positions	AI	avg length
	3-16	0.99	14
	38-49	0.98	12
	DEX0255_154	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
20	22-34	1.07	13
	DEX0255_157	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
	24-45	1.00	22
	5-14	0.97	10
25	DEX0255_158	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
	13-40	1.02	28
	DEX0255_166	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
30	21-30	1.01	10
	DEX0255_168	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
	68-81	1.01	14
	DEX0255_171	Antigenicity Index(Jameson-Wolf)	
35	positions	AI	avg length
	41-77	0.94	37
	DEX0255_175	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
	434-448	1.08	15
40	120-129	0.92	10
	23-87	0.92	65
	DEX0255_176	Antigenicity Index(Jameson-Wolf)	
	positions	AI	avg length
45	5-32	1.11	28

Examples of post-translational modifications (PTMs) of the CSPs of this invention are listed below. In addition, antibodies that specifically bind such post-

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translational modifications may be useful as a diagnostic or as therapeutic. Using the ProSite database (Bairoch et al., Nucleic Acids Res. 25(1):217-221 (1997), the contents of which are incorporated by reference), the following PTMs were predicted for the CSPs of the invention ([http://npsa-pbil.ibcp.fr/cgi-bin/npsa\\_automat.pl?page=npsa\\_prosite.html](http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_prosite.html)

5 most recently accessed October 23, 2001). For full definitions of the PTMs see <http://www.expasy.org/cgi-bin/prosite-list.pl> most recently accessed October 23, 2001.

	DEX0255_105	Ck2_Phospho_Site 11-14; Pkc_Phospho_Site 2-4;3-5;11-13;
	DEX0255_106	Asn_Glycosylation 51-54; Ck2_Phospho_Site 35-38;
		Pkc_Phospho_Site 45-47;
10	DEX0255_107	Ck2_Phospho_Site 27-30; Pkc_Phospho_Site 13-15;
	DEX0255_108	Myristyl 38-43; Pkc_Phospho_Site 14-16;28-30;
	DEX0255_109	Ck2_Phospho_Site 56-59; Myristyl 15-20; Pkc_Phospho_Site 56-58;
	DEX0255_110	Asn_Glycosylation 40-43; Ck2_Phospho_Site 10-13;14-17;19-22;
15		Myristyl 15-20; Pkc_Phospho_Site 79-81;
	DEX0255_111	Pkc_Phospho_Site 8-10;
	DEX0255_112	Pkc_Phospho_Site 21-23;43-45;
	DEX0255_114	Asn_Glycosylation 5-8; Ck2_Phospho_Site 39-42;
		Pkc_Phospho_Site 50-52;
20	DEX0255_115	Asn_Glycosylation 2-5; Glycosaminoglycan 19-22; Myristyl 33-38;37-42;38-43;40-45;49-54;55-60;58-63;64-69;84-89;
		Pkc_Phospho_Site 88-90;143-145; Prokar_Lipoprotein 31-41;
	DEX0255_116	Myristyl 63-68;
	DEX0255_117	Asn_Glycosylation 29-32; Pkc_Phospho_Site 42-44;
25	DEX0255_118	Ck2_Phospho_Site 7-10; Pkc_Phospho_Site 7-9;
		Prokar_Lipoprotein 21-31;
	DEX0255_119	Myristyl 13-18; Pkc_Phospho_Site 21-23;
	DEX0255_120	Asn_Glycosylation 17-20; Ck2_Phospho_Site 52-55;
	DEX0255_121	Ck2_Phospho_Site 13-16; Pkc_Phospho_Site 18-20;44-46;
30	DEX0255_122	Myristyl 2-7;
	DEX0255_123	Ck2_Phospho_Site 46-49;80-83; Myristyl 20-25;43-48;
	DEX0255_125	Camp_Phospho_Site 40-43; Pkc_Phospho_Site 55-57;59-61;67-69;75-77; Tyr_Phospho_Site 68-76;69-76;
	DEX0255_126	Pkc_Phospho_Site 28-30;
35	DEX0255_127	Ck2_Phospho_Site 36-39;75-78; Myristyl 91-96;
	DEX0255_128	Asn_Glycosylation 17-20; Camp_Phospho_Site 4-7;
		Ck2_Phospho_Site 9-12; Pkc_Phospho_Site 9-11;
	DEX0255_130	Myristyl 9-14; Pkc_Phospho_Site 14-16;41-43;45-47;
	DEX0255_131	Ck2_Phospho_Site 13-16;22-25; Myristyl 17-22;
40	DEX0255_132	Myristyl 12-17;
	DEX0255_133	Asn_Glycosylation 33-36;
	DEX0255_136	Ck2_Phospho_Site 22-25; Myristyl 5-10;48-53;56-61;
	DEX0255_137	Amidation 31-34; Camp_Phospho_Site 33-36; Pkc_Phospho_Site 31-33;
45	DEX0255_138	Pkc_Phospho_Site 28-30;

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	DEX0255_139	Pkc_Phospho_Site 22-24;23-25;
	DEX0255_140	Asn_Glycosylation 41-44; Pkc_Phospho_Site 43-45;53-55;
	DEX0255_141	Amidation 274-277;543-546;814-817; Asn_Glycosylation 64-
5		67;294-297;391-394;610-613;611-614;660-663;
		Ck2_Phospho_Site 19-22;114-117;128-131;185-188;263-266;286-
		289;398-401;426-429;532-535;555-558;669-672;697-700;803-
		806;826-829;841-844; Myristyl 89-94;147-152;320-325;345-
		350;446-451;458-463;589-594;614-619;717-722;729-734;
10		Pkc_Phospho_Site 75-77;76-78;102-104;148-150;274-276;381-
		383;459-461;543-545;650-652;730-732;814-816;
	DEX0255_142	Camp_Phospho_Site 18-21;
	DEX0255_143	Pkc_Phospho_Site 16-18;
	DEX0255_144	Ck2_Phospho_Site 24-27;
	DEX0255_145	Camp_Phospho_Site 13-16; Ck2_Phospho_Site 29-32; Myristyl
15		25-30;
	DEX0255_147	Myristyl 43-48; Prokar_Lipoprotein 37-47;
	DEX0255_148	Myristyl 9-14;11-16;32-37;
	DEX0255_149	Asn_Glycosylation 5-8; Pkc_Phospho_Site 18-20;
	DEX0255_150	Asn_Glycosylation 9-12; Ck2_Phospho_Site 23-26;
20	DEX0255_151	Myristyl 68-73;
	DEX0255_152	Leucine_Zipper 52-73; Pkc_Phospho_Site 56-58;
	DEX0255_154	Pkc_Phospho_Site 18-20;
	DEX0255_155	Ck2_Phospho_Site 32-35; Myristyl 28-33;57-62;
		Pkc_Phospho_Site 45-47;
25	DEX0255_157	Asn_Glycosylation 16-19;28-31; Pkc_Phospho_Site 6-8;19-21;25-
		27;38-40;
	DEX0255_159	Ck2_Phospho_Site 27-30;
	DEX0255_164	Amidation 23-26; Pkc_Phospho_Site 33-35;70-72;123-125;
	DEX0255_165	Ck2_Phospho_Site 2-5;42-45; Myristyl 30-35; Pkc_Phospho_Site
30		11-13;35-37;42-44;
	DEX0255_166	Asn_Glycosylation 18-21; Myristyl 25-30; Pkc_Phospho_Site 29-
		31;
	DEX0255_168	Myristyl 91-96; Pkc_Phospho_Site 60-62;
	DEX0255_170	Ck2_Phospho_Site 22-25; Pkc_Phospho_Site 31-33;
35	DEX0255_171	Asn_Glycosylation 141-144; Ck2_Phospho_Site 41-44; Myristyl
		138-143; Pkc_Phospho_Site 41-43;69-71;101-103;120-122;143-
		145;150-152; Protein_Kinase_Atp 127-149; Rgd 49-51;
		Tyr_Phospho_Site 59-66;
	DEX0255_173	Amidation 38-41; Pkc_Phospho_Site 28-30;
40	DEX0255_174	Asn_Glycosylation 93-96; Ck2_Phospho_Site 16-19;46-49;87-90;
	DEX0255_175	Amidation 82-85; Asn_Glycosylation 120-123;344-347;406-409;
		Camp_Phospho_Site 5-8;6-9;486-489; Ck2_Phospho_Site 8-
		11;10-13;28-31;32-35;64-67;70-73;99-102;155-158;267-270;297-
45		300;338-341;404-407;408-411;472-475; Leucine_Zipper 357-
		378;364-385; Myristyl 97-102;159-164;185-190;193-198;
		Pkc_Phospho_Site 4-6;32-34;58-60;211-213;214-216;361-
		363;433-435;

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DEX0255\_176      Amidation 25-28; Camp\_Phospho\_Site 27-30;61-64;  
                          Ck2\_Phospho\_Site 67-70;73-76;109-112;127-130;  
                          Leucine\_Zipper 93-114; Myristyl 29-34;79-84;

5    **Example 6: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide**

RNA is isolated from individual patients or from a family of individuals that have a phenotype of interest. cDNA is then generated from these RNA samples using protocols known in the art. *See, Sambrook (2001), supra.* The cDNA is then used as a  
 10    template for PCR, employing primers surrounding regions of interest in SEQ ID NO: 1 through 100. Suggested PCR conditions consist of 35 cycles at 95°C for 30 seconds; 60-120 seconds at 52-58°C; and 60-120 seconds at 70°C, using buffer solutions described in Sidransky *et al.*, *Science* 252(5006): 706-9 (1991). *See also* Sidransky *et al.*, *Science* 278(5340): 1054-9 (1997).

15        PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations are then cloned and sequenced to validate the results of the direct sequencing. PCR products is  
 20    cloned into T-tailed vectors as described in Holton *et al.*, *Nucleic Acids Res.*, 19: 1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

Genomic rearrangements may also be determined. Genomic clones are nick-translated with digoxigenin deoxyuridine 5' triphosphate (Boehringer Mannheim),  
 25    and FISH is performed as described in Johnson *et al.*, *Methods Cell Biol.* 35: 73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C-and R-bands. Aligned images for precise mapping  
 30    are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. *Id.* Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical

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Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

#### 5   **Example 7: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample**

Antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 µg/ml. The antibodies are either  
10   monoclonal or polyclonal and are produced by the method described above. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced. The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove  
15   unbound polypeptide. Next, 50 µl of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbound conjugate. 75 µl of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution are added to each well and incubated 1 hour at room  
20   temperature.

The reaction is measured by a microtiter plate reader. A standard curve is prepared, using serial dilutions of a control sample, and polypeptide concentrations are plotted on the X-axis (log scale) and fluorescence or absorbance on the Y-axis (linear scale). The concentration of the polypeptide in the sample is calculated using the  
25   standard curve.

#### **Example 8: Formulating a Polypeptide**

The secreted polypeptide composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the secreted polypeptide  
30   alone), the site of delivery, the method of administration, the scheduling of

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administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of secreted polypeptide administered parenterally per dose will be in the range of about 1 ,  $\mu\text{g/kg/day}$  to 10  $\text{mg/kg/day}$  of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01  $\text{mg/kg/day}$ , and most preferably for humans between about 0.01 and 1  $\text{mg/kg/day}$  for the hormone. If given continuously, the secreted polypeptide is typically administered at a dose rate of about 1  $\mu\text{g/kg/hour}$  to about 50  $\text{mg/kg/hour}$ , either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the secreted protein of the invention are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The secreted polypeptide is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semipermeable polymer matrices in the form of shaped articles, e. g., films, or microcapsules. Sustained-release matrices include polylactides (U. S. Pat. No.3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., Biopolymers 22: 547-556 (1983)), poly (2-hydroxyethyl methacrylate) (R. Langer et al., J. Biomed. Mater. Res. 15: 167-277 (1981), and R. Langer, Chem. Tech. 12: 98-105 (1982)), ethylene vinyl acetate (R. Langer et al.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomally entrapped polypeptides. Liposomes containing the secreted polypeptide are prepared by methods known per se: DE Epstein et al., Proc. Natl. Acad. Sci. USA 82: 3688-3692 (1985); Hwang et al., Proc. Natl. Acad.

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Sci. USA 77: 4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U. S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal secreted polypeptide therapy.

For parenteral administration, in one embodiment, the secreted polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, I. e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation.

For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides. Generally, the formulations are prepared by contacting the polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e. g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.



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The secreted polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

5 Any polypeptide to be used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e. g., 0.2 micron membranes). Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

10 Polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1 % (w/v) aqueous polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized  
15 polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container (s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of  
20 pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

#### **Example 9: Method of Treating Decreased Levels of the Polypeptide**

It will be appreciated that conditions caused by a decrease in the standard or  
25 normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a pharmaceutical composition comprising an amount of the polypeptide to increase the  
30 activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 µg/kg of the polypeptide for six consecutive days. Preferably, the

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polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided above.

**Example 10: Method of Treating Increased Levels of the Polypeptide**

Antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer.

For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided above.

**Example 11: Method of Treatment Using Gene Therapy**

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e. g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37°C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks. pMV-7 (Kirschmeier, P. T. et al., DNA, 7: 219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1. Preferably, the 5' primer contains an EcoRI site and the 3' primer

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includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to  
5 transform bacteria HB 101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+aml2 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then  
10 added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media,  
15 containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media.

If the titer of virus is high, then virtually all fibroblasts will be infected and no  
20 selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

#### 25 **Example 12: Method of Treatment Using Gene Therapy-*In Vivo***

Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide.

30 The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known

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in the art, see, for example, W0 90/11092, W0 98/11779; U. S. Patent 5,693,622; 5,705,151; 5,580,859; Tabata H. et al. (1997) *Cardiovasc. Res.* 35 (3): 470-479, Chao J et al. (1997) *Pharmacol. Res.* 35 (6): 517-522, Wolff J. A. (1997) *Neuromuscul. Disord.* 7 (5): 314-318, Schwartz B. et al. (1996) *Gene Ther.* 3 (5): 405-411, Tsurumi Y. et al. 5 (1996) *Circulation* 94 (12): 3281-3290 (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

10 The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P. L. et al. (1995) *Ann. NY Acad. Sci.* 772: 126-139 and Abdallah B. et al. (1995) *Biol. Cell* 85 15 (1): 1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the 20 art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

25 The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide 30 matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by

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the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are

5 differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or

10 RNA will be in the range of from about 0.05 µg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be

15 determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the

20 nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard

25 recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the

30 anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about

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0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e. g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice.

The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

### **Example 13: Transgenic Animals**

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e. g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (i. e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40: 691-698 (1994); Carver et al., Biotechnology (NY) 11: 1263-1270 (1993); Wright et al., Biotechnology (NY) 9: 830-834 (1991); and Hoppe et al., U. S. Patent 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82: 6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56: 313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3: 1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e. g., Ulmer et al., Science 259: 1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm mediated gene transfer (Lavitrano et al., Cell 57: 717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl.

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Rev. Cytol. 115: 171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated  
5 oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380: 64-66 (1996); Wilmut et al., Nature 385: 810813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, I. e., mosaic animals or chimeric. The transgene may be integrated as a single transgene  
10 or as multiple copies such as in concatamers, e. g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89: 6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of  
15 interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal  
20 sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265: 103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell  
25 type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression  
30 of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, in situ hybridization analysis, and reverse transcriptase-PCR

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(rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

#### **Example 14: Knock-Out Animals**

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (E. g., see Smithies et al., *Nature* 317: 230-234 (1985); Thomas & Capecchi, *Cell* 51: 503-512 (1987); Thompson et al., *Cell* 5: 313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such



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approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (e. g., see Thomas & Capecchi 1987 and Thompson 1989, *supra*).

However this approach can be routinely adapted for use in humans provided the

- 5 recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

- In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e. g., knockouts) are administered to a
- 10 patient *in vivo*. Such cells may be obtained from the patient (I. e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e. g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or
- 15 alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e. g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc.

- 20 The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e. g., in the circulation, or intraperitoneally.

- 25 Alternatively, the cells can be incorporated into a matrix and implanted in the body, e. g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U. S. Patent 5,399,349; and Mulligan & Wilson, U. S. Patent 5,460,959 each of which is incorporated by reference herein in its
- 30 entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the

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development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

5           Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

10           All patents, patent publications, and other published references mentioned herein are hereby incorporated by reference in their entireties as if each had been individually and specifically incorporated by reference herein. While preferred illustrative embodiments of the present invention are described, one skilled in the art will appreciate that the present invention can be practiced by other than the described embodiments,  
15           which are presented for purposes of illustration only and not by way of limitation. The present invention is limited only by the claims that follow.

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## CLAIMS

We claim:

1. An isolated nucleic acid molecule comprising
  - (a) a nucleic acid molecule comprising a nucleic acid sequence that encodes  
5 an amino acid sequence of SEQ ID NO: 101 through 176;
  - (b) a nucleic acid molecule comprising a nucleic acid sequence of SEQ ID  
NO: 1 through 100;
  - (c) a nucleic acid molecule that selectively hybridizes to the nucleic acid  
molecule of (a) or (b); or
  - 10 (d) a nucleic acid molecule having at least 60% sequence identity to the nucleic  
acid molecule of (a) or (b).
2. The nucleic acid molecule according to claim 1, wherein the nucleic acid  
molecule is a cDNA.  
15
3. The nucleic acid molecule according to claim 1, wherein the nucleic acid  
molecule is genomic DNA.
4. The nucleic acid molecule according to claim 1, wherein the nucleic acid  
20 molecule is a mammalian nucleic acid molecule.
5. The nucleic acid molecule according to claim 4, wherein the nucleic acid  
molecule is a human nucleic acid molecule.
- 25 6. A method for determining the presence of a colon specific nucleic acid  
(CSNA) in a sample, comprising the steps of:
  - (a) contacting the sample with the nucleic acid molecule according to claim 1  
under conditions in which the nucleic acid molecule will selectively hybridize to a colon  
specific nucleic acid; and
  - 30 (b) detecting hybridization of the nucleic acid molecule to a CSNA in the  
sample, wherein the detection of the hybridization indicates the presence of a CSNA in  
the sample.

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7. A vector comprising the nucleic acid molecule of claim 1.

8. A host cell comprising the vector according to claim 7.

5

9. A method for producing a polypeptide encoded by the nucleic acid molecule according to claim 1, comprising the steps of (a) providing a host cell comprising the nucleic acid molecule operably linked to one or more expression control sequences, and (b) incubating the host cell under conditions in which the polypeptide is produced.

10

10. A polypeptide encoded by the nucleic acid molecule according to claim 1.

11. An isolated polypeptide selected from the group consisting of:

15 (a) a polypeptide comprising an amino acid sequence with at least 60% sequence identity to of SEQ ID NO: 101 through 176; or

(b) a polypeptide comprising an amino acid sequence encoded by a nucleic acid molecule comprising a nucleic acid sequence of SEQ ID NO: 1 through 100.

20 12. An antibody or fragment thereof that specifically binds to the polypeptide according to claim 11.

13. A method for determining the presence of a colon specific protein in a sample, comprising the steps of:

25 (a) contacting the sample with the antibody according to claim 12 under conditions in which the antibody will selectively bind to the colon specific protein; and  
(b) detecting binding of the antibody to a colon specific protein in the sample, wherein the detection of binding indicates the presence of a colon specific protein in the sample.

30 14. A method for diagnosing and monitoring the presence and metastases of colon cancer in a patient, comprising the steps of:

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(a) determining an amount of the nucleic acid molecule of claim 1 or a polypeptide of claim 6 in a sample of a patient; and

(b) comparing the amount of the determined nucleic acid molecule or the polypeptide in the sample of the patient to the amount of the colon specific marker in a normal control; wherein a difference in the amount of the nucleic acid molecule or the polypeptide in the sample compared to the amount of the nucleic acid molecule or the polypeptide in the normal control is associated with the presence of colon cancer.

15. A kit for detecting a risk of cancer or presence of cancer in a patient, said kit comprising a means for determining the presence the nucleic acid molecule of claim 1 or a polypeptide of claim 6 in a sample of a patient.

16. A method of treating a patient with colon cancer, comprising the step of administering a composition according to claim 12 to a patient in need thereof, wherein said administration induces an immune response against the colon cancer cell expressing the nucleic acid molecule or polypeptide.

17. A vaccine comprising the polypeptide or the nucleic acid encoding the polypeptide of claim 11.

20

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Ghosh, Malavika  
Liu, Chenghua  
diaDexus, Inc.

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Genes and Proteins

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 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nggagatgga gtttataagg caccagacc 360  
 agtgcctgat gtccaaactc acttggaat gacgggggac acatctcctt gtttattttt 420  
 atgtttattt attttttttt aatggagctt tgctctgggt gcactccagc ctgggtgaca 480  
 cgacgtgaga ctctgtctca aaaacagact taaaaccctt taaaagtgtg tgataaacat 540  
 cagcttgaca aatatgaacc cagcaccctg gaagcactgg cactatgctg ggtgctgagg 600  
 gagatggcat gaacagaaaa gagagacaca ggcccaagga gggaagtcca gtgtttggtg 660  
 ggagatgggg atgttaacaa tcacaca 687

<210> 7  
 <211> 475  
 <212> DNA



&lt;213&gt; Homo sapiens

&lt;400&gt; 7

```

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tggggcccca gcctggggga gacccgtgac tccaggcatg ctatcaggac aacttcaacc 120
cgagggggaga tcagctctcc cgcaacctgg tgccgctctc tgacagcaca acagaacctc 180
agggggtaca ggatgattca aggaacagtg tgctacagga cctcgggtatt cctgctgaga 240
gtcatttcct gtgctggatt taccatcaca gggaccagat ttatagaaaa atgaacatgt 300
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ctggaacaag agatatagat taagactggt gttacatggt gcctacatga ctttc 475

```

&lt;210&gt; 8

&lt;211&gt; 521

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 8

```

ttttggtgag ccagccagga gtgaagacga caggtttgct gtctcctttg cctgtggggtc 60
tggggcccca gcctggggga gacccgtgac tccaggcatg ctatcaggac aacttcaacc 120
cgagggggaga tcagctctcc cgcaacctgg tgccgctctc tgacagcaca acagaacctc 180
agggggtaca ggatgattca aggaacagtg tgctacagga cctcgggtatt cctgctgaga 240
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```

&lt;210&gt; 9

&lt;211&gt; 743

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 9

```

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taattacggc tggaaaagat ttaaactatt agaatagaga agactggatt agagaatatt 120
aatattctag aaaataacaa gcttatgaca ggaatactat atcagagtca agagaaaaca 180
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agtcaacagt atttattgag tccctactat gtgtgccaaa ataaaaaatt aactaagtac 300
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tgctgtgcca agaaagcctc tttggaggcc attgccataa tctactgttt acatttgtgc 660
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ctatttcagt tcccctatcg act 743

```

&lt;210&gt; 10

&lt;211&gt; 548

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 10

```

atgtaaaggc ttaaactcagc acctggacta catatataag gtgatcaata aatatctact 60
ttgggtatctg aatgcttcaa gatagccttt tgttcccttc taaaaaagag tgggtagtga 120

```

```

ttgttaagac ttacccgctc ataaatgttt tattacatac ttaatatgtg aagctatcaa 180
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ccataatgcc aacttttctaa gcttaagctg actgtaacaa agaactggct ttgtaacaaa 300
gaacaagtct tactggcttc atgcaatgca aacatTTTTT tcttacgtat ctgacagatc 360
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cacattctat cacgaggctt agccatattg gagtttttca cttcgggtgat gaggatgagg 480
gagaaagtgg gagagagtga aaagaggggag aaaggtggaa aaggggagaaa gaaggagaag 540
gtggaaaag                                     548

```

&lt;210&gt; 11

&lt;211&gt; 797

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 11

```

actggccttag cacttacttc ttggtgtgaa gagctagttc ttccctagag agcccaaact 60
ctggagccag gtggattgag tgggaatcct agctctgcta aaggacgta cttgcctaag 120
tctcctctc agtaaataagg atactagtga taacatcttc atagagtgtg catgagaatt 180
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cccaaatagg aaaccataat gccaaacttc taagcttaag ctgactgtaa caaagaactg 480
gctttgtaac aaagaacaag tcttactggc ttcattgcaat gcaaacattt ttttcttacg 540
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gatgaggatg agggagaaaag tgggagagag tgaagagagg gagaaagggt gaaaaggagg 720
aaagaaggag aaggtggaaa ggagagagag agaaattgag aaagtgagaa aaaaaaaaaa 780
aaaaaacaaa aaaaaaa                                     797

```

&lt;210&gt; 12

&lt;211&gt; 558

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 12

```

ccatatcttg tttttgacca gtgaagggga aagaaagaaa gtaattctag aacaacctac 60
tacttacttt agagcttcac ttttatatca tctatatttg acttggaac catctgtgtt 120
tttgtctttg tggtcagcca ttattgtttg ttttgccatg atatatgtcg gaatgatatt 180
atttgactct cctttaagta cctcctgctt ctttgagtaa ggagatccta ccagtatcta 240
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ccaaaacact gaaactccct ctacttatga aataaacaaa ctggcttaaa ttgggtggaa 360
tcattatggc caaatggtgt ttgggcagaa tcaccttgct gaggtcaccg tctgaatttt 420
cactgcctgt ttcattcaca ctccccttca gtttgcccat gacatccatg aggaggcaat 480
gagcttgaga gtaacgtttc agtataaaat ggctttctct taaaaacctg atgtcatagt 540
atgggctact agcacttc                                     558

```

&lt;210&gt; 13

&lt;211&gt; 596

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 13

```

aaagatgaca tggattagct tcaagtaaca cataatgtgg ctggagtcag ctgatcttta 60
tgctgaaagt gtcaacagta gtgacaaata cttcaagtaa cgggtcaaaa gtctaagaca 120
gtcattctgc cagaaatggg ctgggacttc cccatacatg ggacacgtag atcaactttc 180

```

tccaagaacc	accaacctgg	catgcagtga	tgacctctgc	agtagacagg	gatttaggct	240
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tgacaattag	atcagcagct	aagataaatt	tcagtttgca	gccccaaaga	agatgttctt	360
aattagacag	ttaatcattt	tcaatatgga	agcctaaaca	cgtagaatgt	ggcaataacc	420
cggaaactctc	agttctgaca	attgagtga	gtaatcaccc	cagattcagg	ttcttcattg	480
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tggcccatta	gtgaaccagg	aacagatat	tagaaaatat	tcagtaaatt	ggggag	596

&lt;210&gt; 14

&lt;211&gt; 1114

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 14

gtagacattt	aagtttttaa	tcctgtaaag	agaagcagca	gagatgcaac	ataggctcag	60
actttaagca	gctctaattt	catggctttc	ctgggcctcc	accatattgc	catagggcct	120
gtcacatgct	tatgcacct	actggctgca	gcagtgcac	ctgaagacag	ctcagtatag	180
ccatgggcta	cttttctagt	catgggctgt	ggaacctgga	acatgtgacc	agaataccac	240
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cctcaccagc	cagaagggga	tgtatgggg	aagcagaacc	ctattagaga	gaaaagccaa	360
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ctctgtgtcc	tgatgttaaa	cttaaaatga	aata			1114

&lt;210&gt; 15

&lt;211&gt; 1185

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 15

gtagacattt	aagtttttaa	tcctgtaaag	agaagcagca	gagatgcaac	ataggctcag	60
actttaagca	gctctaattt	catggctttc	ctgggcctcc	accatattgc	catagggcct	120
gtcacatgct	tatgcacct	actggctgca	gcagtgcac	ctgaagacag	ctcagtatag	180
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gttctaaggt	atgcactatt	ctccccctca	gcacagccct	ttgggccttt	tacacaatgg	540
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ctctgtgtcc tgatgtttaa cttaaaatga aatagttttc agaataaaaa taaatttgta 1140
tatattaaaa aaacaaaaaa aaaaaagatc ttttaattaag cggac 1185
```

<210> 16  
 <211> 413  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> unsure  
 <222> (138)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (145)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (151)  
 <223> a, c, g or t

```
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aagtaattta ttatttttnat ttatnattat naccattatt attaacaact ttgtgaacaa 180
aagcatttat ttaaatgttt tatgggttatt tgtattctct atttgtagga ttcaaagttt 240
tatattcaac ttattgagat tattatagaa atgaggtata tttgagaggg tcaaattattc 300
taaaagaata tttcagacac cacctcctcc agcaacaaaa atagctacaa accaatataa 360
attcatgtta gttattttca tacatattga aaactttact gtacattaaa agg 413
```

<210> 17  
 <211> 517  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> unsure  
 <222> (138)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (145)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (151)  
 <223> a, c, g or t

```
<400> 17
acaaaatggt tttatgtgtt taggtctgac ggtatgtctt cttattgttg actttttaa 60
ttattaaatt gtagtttagg agaatgagct aatataatct ctaatagctt agaataattg 120
aagtaattta ttatttttnat ttatnattat naccattatt attaacaact ttgtgaacaa 180
```

```

aagcatttat ttaaatgttt tatgggttatt tgtattctct atttgtagga ttcaaagttt 240
tatattcaac ttattgagat tattatagaa atgaggtata tttgagaggg tcaaattatc 300
taaaagaata ttccagacac cacctcctcc agcaacaaaa atagctacaa accaatataa 360
atttcattgtt agttattttc atacatattg aaaactttac tgtacattaa aagggtgtac 420
agacagccct ccatactctgt gggtttcaca tccatgtatt caaccaagca tggatcaaaa 480
atattcagta aaaaaaaagt cgacgcggcc gcgaatt 517

```

&lt;210&gt; 18

&lt;211&gt; 502

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (292)

&lt;223&gt; a, c, g or t

&lt;400&gt; 18

```

cacacagaat gcacaaaggc agggctgctc ttttattttg ggtggcgtgg gtggaagtag 60
gcaatggagg cacagttgtc aaagccactg gtctaatacta actattcttc tctctccac 120
tttacagagg gtgtaacttg ggccaagaaa tcgtgatgtg agtaggtcac acagtgttga 180
gggccaaaaac cctcgactcc gttgggagtt aaaggggagg aagtggacat ttcctttgaa 240
cctaataata agcaggtgag gttttgtatt ctcatTTTTc acagtgcaag anaattttgt 300
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ccattactgt aaactccatc tctccgctgc tgtcagctcc ttcttgatcc gtacagaaat 480
aaaagtttgc caccaaaaaa aa 502

```

&lt;210&gt; 19

&lt;211&gt; 2961

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 19

```

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gaaggtcagt cgtggcctcc ttccagaagg aaagagattt tagcaggaa agacaggcaa 480
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cccttggtaa gaatgctgca gctgggggtca gtctccaaac tggagtttgg tggctaaaaat 1560
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```

&lt;210&gt; 20

&lt;211&gt; 653

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 20

```

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agccaggatc agcatgggaa caatcaagga ggcaggggct tatcagtact atggaccctt 120
acactggctc tgcctgggtg ttcttctctt cgcataccaa agacagaaat taagcctcca 180
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&lt;210&gt; 21

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&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 21

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10

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&lt;210&gt; 22

&lt;211&gt; 148

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (9)

&lt;223&gt; a, c, g or t

&lt;400&gt; 22

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caaatgcttt taagatattg tacatctg 148

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&lt;210&gt; 23

&lt;211&gt; 398

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 23

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&lt;210&gt; 24

&lt;211&gt; 523

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 24

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 <213> Homo sapiens

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 <222> (5780)  
 <223> a, c, g or t

<220>  
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 <222> (5885)  
 <223> a, c, g or t

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 <213> Homo sapiens

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 <222> (502)..(565)  
 <223> a, c, g or t

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 <213> Homo sapiens

<220>  
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 <222> (175)  
 <223> a, c, g or t

<400> 28

14

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ggttgacatt t                                     191

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&lt;210&gt; 29

&lt;211&gt; 998

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 29

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&lt;210&gt; 30

&lt;211&gt; 282

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (5)

&lt;223&gt; a, c, g or t

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (17)

&lt;223&gt; a, c, g or t

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (29)

&lt;223&gt; a, c, g or t

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (110)

&lt;223&gt; a, c, g or t

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (128)..(217)

<223> a, c, g or t

<400> 30

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<211> 1225

<212> DNA

<213> Homo sapiens

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<210> 32

<211> 844

<212> DNA

<213> Homo sapiens

<400> 32

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tgttttgtaa taattaaact tgtaattatt tgacttgaaa tatttaatca tttttttggg 300
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aaat

844

&lt;210&gt; 33

&lt;211&gt; 2483

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 33

```

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ccaaggccac cccgaacaca tcttttccct tccctaaat aaattctact ggattcttct 960
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2483

```

&lt;210&gt; 34

&lt;211&gt; 591

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 34

17

```

aatccattta aagtcaacta aatgttattt gggtaatatg tccctcatgt taaatttgcc 60
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ctttctcagg acaaactcga gcttattaga aaactaggga gtgatctggt g 591

```

&lt;210&gt; 35

&lt;211&gt; 306

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 35

```

cctagggaga atcagcaagg aaaagatcaa tgtaatcttg aataacttat cctgaaactt 60
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agtaccagtt taacacataa aaagtgatca aggtgcaagg gacacagctt tgaaatagtc 180
agacctggat ctgaatctgt gattctgtca tctgcaataa gtttctaact tctccaagcc 240
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ttactc                                           306

```

&lt;210&gt; 36

&lt;211&gt; 617

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 36

```

ccaagactga gttagatttt ctattatgta ctcccatggc aacagcattt tccacttaac 60
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tactgacata ctgaaacaca tattaatata accaaaaata aaaatttcat gagacagtat 600
taatgtttac cacatgc                                           617

```

&lt;210&gt; 37

&lt;211&gt; 725

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 37

```

ccaagactga gttagatttt ctattatgta ctcccatggc aacagcattt tccacttaac 60
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ccaatgtttt tcaggttttt tactaggagc acatgcatga atgtgtatat atgtgtatag 480

```

18

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ctatgcaaaa acatgaacag atgtatgcat gtgtataatc taaaacacat aaaggtacat 540
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taatgttaac cacatgctat atacttataat ttttctttca tttgcaaaag aatgctgtta 660
tgactgtcta aacctctggc ttgagaaaaa aaaaaaaaaa aaaaagatct ttaattaagc 720
gtgcc                                             725

```

&lt;210&gt; 38

&lt;211&gt; 90

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 38

```

gtaaaatatc tgtctcactg gcaatttttt ttacattgaa tttgttgaca attttttttac 60
attgaatatg ttaaaatttt tatatatattg 90

```

&lt;210&gt; 39

&lt;211&gt; 222

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 39

```

tgtagagatg ggatctctct ttgttgcccg ggctggctctg gaattctctg ggttcagggtg 60
atcctgctac gtcagccatg agccacggtg cccagcctgg caggcttggt ttctcttaaat 120
gcctctcctt ggcttgcaag atggccacct tctggctgtg tcctctctct catggccttt 180
cctttgtggg cacacatcct tgttctctcc ttctctttat aa 222

```

&lt;210&gt; 40

&lt;211&gt; 257

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 40

```

gtttttcccat tgactaacgc ttaagatata ttggagtcaa atgctcataa aatgctcatc 60
caatgcttat aaaatattag agttgaaatg gactctctgt tcatgcagat gatgagaccg 120
aaacagagag cttccaggag gatcaatgcc attcaatgag cttgctgctg tactcccctc 180
tacacaatat ggatatatcc catcccagcc cgagactggc catactagtt ctagtaactg 240
aggttttccct cctactt 257

```

&lt;210&gt; 41

&lt;211&gt; 263

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 41

```

gctcgagggt ttccattga ctaacgctta agatatattg gagtcaaatg ctcataaaaat 60
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agaccgaaac agagagcttc caggaggatc aatgccattc aatgagcttg ctgctgtact 180
ccctctaca caatatggat atatcccatc ccagcccagag actggccata ctagttctag 240
taactgaggc tttcctccta ctt 263

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&lt;210&gt; 42

&lt;211&gt; 533

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

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 <222> (501)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (514)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (528)  
 <223> a, c, g or t

<400> 42  
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 atacaataga aagatcctgg aatcccgaca tgaggacaaa aatggtactg aattcttttt 180  
 gaaaaataga ttactgaaaa gcgatctaata atagaacagt tgctttttact tagatgttca 240  
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 ttcgttatga tataatactt tctattgtaa actggactaa agaaacgttg tatgttcaag 360  
 gaagtgttga gcagccatgg tgttcctggg acatgctccc cagggtgctga gagaggtgct 420  
 gcaggagtca cagacctgca ggcacgcact tgccagtgcac tgggacgttg gctggtggtt 480  
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 <212> DNA  
 <213> Homo sapiens

<400> 43  
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 gaagtgttga gcagccatgg tgttcctggg acaggctccc cagggtgctga gagaggtgct 420  
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 ctctcaagcc ttcgagtgga tgctgtttca tatttcaccc agcctgggag ttggagacct 660  
 gagctgcatt acctaa 676

<210> 44  
 <211> 251  
 <212> DNA  
 <213> Homo sapiens

<400> 44  
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 taatgctact gaacagctac agagcactcc tctgaactca ctggaatggg ctatatccca 180  
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 agaaatcccg a 251



<210> 45  
 <211> 606  
 <212> DNA  
 <213> Homo sapiens

<400> 45  
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<210> 46  
 <211> 455  
 <212> DNA  
 <213> Homo sapiens

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 <222> (14)  
 <223> a, c, g or t

<220>  
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 <222> (16)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (18)  
 <223> a, c, g or t

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 atgaagccat ctggttaagt atttaaaagt tcatt 455

<210> 47  
 <211> 367  
 <212> DNA  
 <213> Homo sapiens

<400> 47  
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21

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tggggca 367

```

&lt;210&gt; 48

&lt;211&gt; 249

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 48

```

gttttattgt tttcatgcat tcttaggtct tctactgcat gcttcctaaa aacaggggtca 60
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gggcctgccg gggaaataaa cttggttgag acaaaattct tgtaaataag ctcatagagg 180
ggacagactc ctgctccatt cctcccaccc ctcaacaagg cttccaaatt agcggaaaac 240
agtctaaat 249

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&lt;210&gt; 49

&lt;211&gt; 436

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 49

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aaaatgacct tcttttgctg ttttctgac cacaagggtg attctgcccc tcagggcctc 60
cctttacctt ggcacacaag ggctccatc ctggggcacc ctcccacctg cccaccct 120
ccaacctaac tacttgccat ttcccaaata catcgtgcag tgccctgggt ctatcctgtg 180
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ggatgccatg ttgctt 436

```

&lt;210&gt; 50

&lt;211&gt; 853

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 50

```

cctggataat aaatgcctaa ctctcaccaa ctaccttgcc atcatgggct tcttcgatca 60
gaaatctagt aagagagtat ggtgggggct gcgagacca tcatcactac caaaaaacat 120
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ctggggaatt ttactttttg ctggaaaagg agtcaccctc ccttgccaac cacatgtgtg 780
gttatacatt ggtattgcag agtgatgcca tttaacaagta atacatttga gttggcagat 840
ttcccaaggt ttc 853

```

<210> 51  
 <211> 383  
 <212> DNA  
 <213> Homo sapiens

<400> 51  
 tgaaaattga ccaacaacca gaagtgacag caacaacaaa ataccaagtc agtaaagatg 60  
 gagagaaata gggagcagtg gaaggtagat gtcatttctg ttttttagtgg tggaatacaa 120  
 ggtgttcttg tgcttaaagg tcatgttctt gtgataaaac gcaactgcaga gacaacatag 180  
 ttttaattggc tgaggcaggt gactcccttt aagcatcagg gtggaacaaa ctacacgaca 240  
 aaatgtaatt ttaaaaacca ctctcattca aatgtaagaa tatcaaagca cccttaactc 300  
 attacatgag tgaacaatg agtgtcatgg tctgaattgt gttccctctc ccaaaccctg 360  
 atgttcaagc cttaaaccct agt 383

<210> 52  
 <211> 3342  
 <212> DNA  
 <213> Homo sapiens

<400> 52  
 tgaaaattga ccaacaacca gaagtgacag caacaacaaa ataccaagtc agtaaagatg 60  
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 gtgttcttgt gcttaaagg tcatgttctt tgataaaacg cactgcagag acaacatag 180  
 ttaattggct gaggcagtag ctactgcacg acccccacg tccgcctcct gccattgcca 240  
 gcaggtgcct tgcgcggta cctggctgcg cttattcatc cattatggtc gctctgtcac 300  
 tgggtgccatt atgtgctcac atgcccactc cctcaggttt agaagtcgcg ttgcccggca 360  
 acagaacaat ctgctggctt agcctttggc caagttggca gctggacgag gacgctcaga 420  
 gccagctct tgagagttca agtatccgac agttccccac tgctcccagg agcggttacc 480  
 cgggcactct gtgcccctca ttctgttttg ggccaaggcc gaggacctgc gactaccaca 540  
 gtaagccagt gctgtgtgct ccgagttcca ggcatcccc cagctcagcc actacactga 600  
 gcacaaggac tctgtggggc ccaggagcag gtagtcaccc ctttggggtc cacaacaccc 660  
 ggctgtcccc agacttgtgt ccagggaaga tagtgttgag ggccctcaag gagagcgggg 720  
 cagggatgcc tgagcagcac aaggaccca gagtccaaga aaatcctgat gatcagagaa 780  
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 cccccagatc ccaggcctct agggccaca tcaacactgc actgcacgtt gaggacaagg 2160  
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```

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cagctcccggt gtctgggaag aagcacagac caccaggacc cctgttctcc tcctcagatc 2400
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gggactcagc ccaggacacc tcgctgattc ctgccccctt cacacctgca agcagggatg 3300
ccggcatcag aagaatgttt cgtgttcgaa attgtttgag gg 3342

```

&lt;210&gt; 53

&lt;211&gt; 129

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 53

```

agctgtcata cttatcgttg ctgcttatta gtatttttat ggtttgttat ttcaaaagaa 60
attcatttcc cataacaata ttattttctt aaatatgtta agctttaaaa taaaagcata 120
tcaaatgga 129

```

&lt;210&gt; 54

&lt;211&gt; 201

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 54

```

catgccgtgg cccatgccca tttgcacagg gacgcagggg gttctcacac acaggcaggg 60
tccgccccca gctgccgtcg gcgtcagtc acacacatag gcttttgggc ggtgctggaa 120
gcttctggcc cctgaacgtt cccccaggc cccgtttcca gggaaaggga taggcaggcg 180
cacgtcgcg ccgtttccac a 201

```

&lt;210&gt; 55

&lt;211&gt; 227

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 55

```

catgccgtgg cccatgccca tttgcacagg gacgcagggg gtctcacaca caggcaggg 60
ccgccccag ctgccgtcg gcgtcagtc cacacatag cttttgggc ggtgctggaag 120
cttctggccc ctgaacgttc cccccaggc cccgtttccag ggaaaggat aggcaggcgc 180
acgtcgcggc cgtttccaca atccgacct gtagctgggg cgtgccg 227

```

&lt;210&gt; 56

&lt;211&gt; 271

&lt;212&gt; DNA

<213> Homo sapiens

<400> 56

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catcttttta atattcagta tgaccgaata aagcactggg gctgccttag taacaatggg 60
tgtntctcaag gtaaaacttct catgtgcttg tttcagttgt gagctcaatt agcctctttc 120
tcatgaaatg aatgcctttt tacttgaaag aatgactgag agccaggcta tggatattca 180
aacatgtatt tttcagacac ttcttgaaaa taagtgaagc aaacctgtta attacaaggg 240
aagcaatgac aatatttgtt gccaatgata a                                     271
```

<210> 57

<211> 573

<212> DNA

<213> Homo sapiens

<400> 57

```
gggcaagaca gctgtgggag ttgttgggtc cagtatcttt ttcttccttt ccggatggga 60
agagtgcata tctcttggcc cacaaatgtg tttgcacact aaggatgatg catccttaga 120
aggaagcaga gcagtgcctc gaccttcgct tctggaaccg agaaaatgat gccatgctgc 180
tttgttgggtg tgattgttgt tggttttttg tggatgaatt ttaaaatagt atttgtgact 240
atcatttcat gtgtccactc tttttaaaaa tgttaccttt tctaggattg gcagaatttg 300
gaattatatg tcttattaaa tatgctttga aagacagaag taataagtgc tggttaatct 360
tttatagtgt ttgtcttggg agcaaatagt atgagagaga ggtgtgagaa tgggaataat 420
aatctaacat atcaaaatta gagaacccca aaccatcaca ttctttctct ttgtgccatt 480
ttagaattga gaataccgtc cttcttactg tggttatatt tttacttttg tatataaact 540
tgtagcagaa aataagattc agtagcttaa agg                                     573
```

<210> 58

<211> 843

<212> DNA

<213> Homo sapiens

<400> 58

```
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gagtgcatac ctcttggtcc acaaatgtgt ttgcacacta aggtgatggc atccttagaa 120
ggaagcagag cagtgcctctg accttcgctt ctggaaccga gaaaatgatg ccatgctgct 180
ttgttgggtg gattgttgtt gggttttttg ggatgaattt taaaatagta tttgtgacta 240
tcatttcatg tgtccactct ttttaaaaaat gttacctttt ctaggattgg cagaatttgg 300
aattatatgt cttattaaat atgctttgaa agacagaagt aataagttct ggtaaatctt 360
ttatagtgtt tgtcttggga gcaaatagta tgagagagag gtgtgagaat gggaataata 420
atctaacata tcaaaattag agaaccccaa accatcacat tctttctctt tgtgccattt 480
tagaattgag aataccgtcc ttcttactgt ggttatattt ttacttttgt atataaactt 540
gtagcagaaa ataagattca gtagcttaaa ggggccaggc actgtggctc atgcccgtaa 600
tcccagtggt ttagaaggct gaggggaagg atcacttgag gcctggagtt tgaaatcaac 660
ctgggcaaca tagcaagact ctgttccttc aaaaaaaaaa ttttaaaaaa tagctgagca 720
tggaggtgca tgcctataat cctagcaatg attataccat tacactccag cctggatgat 780
agagtgcac cctgtctcaa agagaaaaaa aaaaaaaaaa aattctgcgg cgcaagaatt 840
cgc                                     843
```

<210> 59

<211> 221

<212> DNA

<213> Homo sapiens

<400> 59

```
cgggactgga aggggtgtgcc ctgcgcgtcc tcgccttcgt cttgcacggg acaagatgtc 60
acgattccga atccaaacct cagagacagc ccccatccct ctcgttagcc acccacacac 120
```

25

```

cccgcctcagc aacaataaca acctgcattt agggaaacgtg tggtatgtgc caggccacac 180
aggcattatc tcatgtactc ctcacaggca ctttatcaag g                221

```

```

<210> 60
<211> 535
<212> DNA
<213> Homo sapiens

```

```

<400> 60
gcacgtgggg tcggggtggg ggcgaagggc cgcttggcct ctgtagggtc gggactggaa 60
gggtgtgccc tcgcgcgtcc cgccttcgtc ttgcacggga caagatgtca cgattccgaa 120
tccaaacctc agagacagcc cccatccctc tcgttagcca cccacacacc ccgctcagca 180
acaataaaca cctgcattta gggaaacgtg gttatgtgcc aggccacaca ggcattatct 240
catgtactcc tcacaggcac cttatcaagg agatgctgtt gttacctgca ttttacagat 300
ggggaaactg aggtcfaatg cattaaggac tgccagggaag ccctgtcctg tggctgtgat 360
gatggaaatg ttccctgtgt tgttcagtat ggtagtcaat ggccacaagt gagcactgga 420
aatgtgccta ttgagactga ggaactgatt ttttcatttt gtttaattgt aattaaacag 480
ttacgtgtgg ctgtggtatt ggaaaaaaaa aaaacaaaaa aaaaaaaaaa aactc      535

```

```

<210> 61
<211> 514
<212> DNA
<213> Homo sapiens

```

```

<400> 61
cgtctcactg atatccctt cagttctccc aaatcacctt ttctgaaaca tacatccgat 60
catgtcattc ctttgcaaaa ctaagtttcc ctttgcactc aaaacaatat ctgaatgtct 120
tgctctggtt tctcaggccc cgcctctacc actggcctca gctcttcccc tctctccatt 180
gctcactgaa taacagccac caagacctcc ttgccattgc tcaaaccatgc aaggcctaca 240
cctgccacag ggccttggca catgctattc catctgttta caatgcttgt ctccacatgg 300
ctacttcttt gtacgagttg gtctcagctc aaatgtcatg tccccaaacca gcctacctaa 360
agcagttctc cctacctagg cttttcttgc tcaccatgta aaagattcct atttagtttc 420
tggtattatc cttcttgctc tagaatggaa gccctacgag ggcaagatat ttttctgtat 480
cggtcactgc tatagcttca acaccaagaa catg                514

```

```

<210> 62
<211> 598
<212> DNA
<213> Homo sapiens

```

```

<400> 62
tttcttctgg tcttattcct ctggatctaa atatatcaac attgacatgt gaggtcagaa 60
ttttatcatt tgaaaaattt ttttcacttt actgaaaatt cagtcaattc ttctgaaga 120
aggaagcaat tgcatatcaa ttttcttcta ttccagctta atctatttat ttttctcttt 180
tacattaaaa cattctttta atgatatatg ctgcctgtaa atatttccca cccactttcc 240
agaggtaatc cactgttatc aagtaagttt agtaaathtt ttttaattga attttctcaa 300
taggtcatta acgtgtttca aagttgaaaa attacaaaac tatgtgtcgt gaaaagtctc 360
cttctttccc ttgtgtccca agctacctag ttcttgagc cagttgatgt tatcagattc 420
tttggtattc tttcagacac acatgggatg cattatttga gcaaaggggc gtgggtgtgt 480
gtccctctgt ttttaagttc taaatgtag catgctacac atacttttt catatatatt 540
cttaagtaac ttcatttcat tatatgtatt ccactttgta aaattagata ctacatgc 598

```

```

<210> 63
<211> 648
<212> DNA

```

&lt;213&gt; Homo sapiens

&lt;400&gt; 63

```

tgaatttctt ctggtcttat tcctctggat ctaaatatat caacattgac atgtgagggtc 60
agaattttat catttgaaaa atttttttca ctttactgaa aattcagtca cttcttcctg 120
aagaaggaag caattgcata tcaattttct tctattccag cttaatctat ttatttttct 180
cttttacatt aaaacattct tttaatgata tatgctgcct gtaaatattt cccaccct 240
ttccagaggt aatccactgt tatcaagtaa gtttagtaaa ttttttttaa ttgaattttc 300
tcaatagggtc attaacgtgt ttcaaagtgt aaaaattaca aaactatgtg tcgtgaaaag 360
tctccttctt tcccttgtgt cccaagctac ctagttcttg gagccagttg atgttatcag 420
attcttttgt attctttcag acacacatgg tatgcatttt tgagcaaagg ggcgtgggtg 480
tgtgtccctc tgtttttaag ttctaaatgt tagcatgcta cacatacttt tttcatatat 540
tttcttaagt aactttattt cattatttgt attcagtttt gtaaaattag atactacatg 600
catgtgggtc aaaagtaaaa tgatgtaaa gctaataata tgtaatat 648

```

&lt;210&gt; 64

&lt;211&gt; 601

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 64

```

gaggttaagcc cattctcacc ctacaggcag ggtagggagg ggcacattct gagaagtggg 60
tatcacaagt tgtccctcat gtggatccca tgacttgagt agtgggaaac tccaagctga 120
tttgaagatt ctttatattga gagtggacct gcacacctag tgttcctggg tcagtccagg 180
ggcgagcaga tcattgaagg actgcacctt tatcctaggc tcaccaaata cccaggtgta 240
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gcatggaatg aggaccagca ggaagagatc tcagaaaaca taagataatg gacttggtga 360
catagatttt aaaggcctta ctcaaactaa ataggcaaga ttgaaaatac ctatgaggaa 420
gaggaaacta tacaatgacc tagcaaattt gaaaaaggaa ccaggaacaa cttgtagaca 480
tgaaaagttc atgtctttat aataaaaaatc taacagatgg atttactagc agattacata 540
aaactgaaga gagtgaatga cctggaaagt agagaagaag aaatataatt tagagaacca 600
c 601

```

&lt;210&gt; 65

&lt;211&gt; 1216

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (58) .. (125)

&lt;223&gt; a, c, g or t

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (1204)

&lt;223&gt; a, c, g or t

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (1206)

&lt;223&gt; a, c, g or t

&lt;400&gt; 65

```

tctcctgate ttggacatca gaactcctga ttctcaagcc tttgggtttg gactggannn 60
nnnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 120
nnnnntaact ctaatacacc agtacaatgg aaagattcct aaattcaaaa gccagaaggc 180

```

tgggttcctg	ttcccaccct	gccttttacc	ttctgtgtgt	tcctgatgaa	gacacttcat	240
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tgcttgagg	gactcagagg	ctgtggagag	tgaatatttc	catcagctga	tgcccttctc	360
aggcctcaat	ctccccctgt	ttgcagactg	tgccctctct	tgccctctgt	ggaatctggc	420
catttgaatc	ctgtcagccc	tgttttccat	caccaaagga	ctccggagga	actgtgccaa	480
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gtccattccc	ttgaataaact	ctgctaatat	ttgaaaatta	gttcccttgc	tcctgatcat	600
gctactgggt	atttggatat	aagagccaag	gatgagggca	atagaaaatt	aaaatcatgt	660
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tccccctacc	ttcctcagaa	cctgctacaa	ggaaagattt	atagactcga	aagcgtcaat	1140
gactgattag	accatatga	ttgctcctgc	tgtttctgat	attttaaaaa	attgtcttat	1200
aaangnataa	aaataa					1216

&lt;210&gt; 66

&lt;211&gt; 1430

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 66

gctcaccaat	gtgggtgggc	ctcattcaat	ccattgcaga	cttgaataga	actaaaagga	60
agagggaagg	caaatttgtt	ggctgcttga	gctgggatat	tcattcttct	cctgatcttg	120
gacatcagaa	ctcctgattc	tcaagccttt	gggtttggac	tggaggcacc	agctttcctg	180
ggcctccagc	ttgcagatgg	catatcatgg	aacttctcag	cctccaaatt	cataactcta	240
atacaccagt	acaatggaaa	gattcctaaa	ttcaaaagcc	agaaggctgg	gttctctgtc	300
ccaccctgcc	ttttaccttc	tgtgtgttcc	tgatgaagac	acttcatgct	ccactattta	360
cttacctctg	aaaogaagg	ctgaccagga	tcagttgttc	tctgacctgc	ttggaggggac	420
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ccctggtttg	cagactgtgg	ccctccttgg	cctctgtgga	atctggccat	ttgaatcctg	540
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cacgggcaag	ctttctgaaa	aagacgtgcc	aactgcagcc	acagaaagtc	cattcccttg	660
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tggatataag	agccaaggat	gagggaataa	gaaaattaaa	atcatgttct	actcatataa	780
actgcacaga	tatggaagg	taggtcctat	tacctataat	cctgggattt	ttagactctc	840
actttcattg	gaccagagtt	gccttaggga	cagtaaaaaa	acaaaatgct	gggtattgtt	900
ttcatcaagc	aactactgat	agtgcacatt	taaatcaaaa	ttcttctaata	cccaaactca	960
gtaaacagat	gctgtgagct	tagttctgcc	cctctggctt	cagattttac	cccactggat	1020
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aacaacagct	ttggactaaa	atatgaagg	gttgttttcc	ttcaatctcc	ccctaccttc	1200
ctcagaacct	gctacaagga	aagatttata	gactcgaaag	cgtcaatgac	tgattagacc	1260
catatgattg	ctcctgctgt	ttctgatatt	ttaaaaaatt	gtctcataaa	gagatacaaa	1320
taaataatca	atggcaaaact	tctggcatgg	gagagacatt	tagggaaaga	agtcattctca	1380
gcctcccca	acacacacat	gcacacacac	atacagctgc	aaacacaatt		1430

&lt;210&gt; 67

&lt;211&gt; 430

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; unsure



&lt;222&gt; (72)..(139)

&lt;223&gt; a, c, g or t

&lt;400&gt; 67

```

gggatattca tctttctcct gatcttggac atcagaactc ctgattctca agcctttggg 60
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nnnnnnnnnn nnnnnnnnnn aactctaata caccagtaca atggacagat tcctaaattc 180
taaagccaga aggctgggtt cctgttccca cctgccttt taccttctgt gtgttcctga 240
tgaagacact tcatgctcca ctatgtactt acctctgaaa cgaagggtctg acccagatca 300
gttggttctct gacctgcttg gagggactca gaggtgtgg agactgtggc cctccttggc 360
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gaggaactgt . 430

```

&lt;210&gt; 68

&lt;211&gt; 829

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (240)..(354)

&lt;223&gt; a, c, g or t

&lt;400&gt; 68

```

gtatgtacta cccacataag tgggacactt tgaacaatga aatatagatg ttttcaccaa 60
agaagggagt cttatttttt tccgacttca gacaattcat cttcatccat taatttttcc 120
tttttgtaat atgtaccttt atgctaattt ttaatatgca aataacttac aaatatatgc 180
tcagcatttg agtacaggct gtgctttatt acatattaca tgcattgtat caatgtactn 240
nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 300
nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnacaaaa 360
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tgaaacacct catatcacca ccaactgtga gccagatata atagactgta ctgtgtaagg 660
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ttaatgctga attataagca aaacctacaa gaataaaatc attttatgct ttgaaactga 780
ctcctttttt aaaaaaagaa tgatcacaaac taccaactcc ctcatctat 829

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&lt;210&gt; 69

&lt;211&gt; 541

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 69

```

atagactagt aaagtctgtt tttatataaa agtgacacag gaagctgtta caatctagga 60
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agatcctaac agagtgcac ttgtgctttt cctaacagac ctgtcggact ggctttttct 180
cttttaagga tatagagaaa gcaaaattag caaatctagt ttcttgtcac ttacttagga 240
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ttcagatgca gcccggagat gtaaccctga ggctggagtc ctgtggctct aatcccagac 480
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a 541

```

<210> 70  
 <211> 696  
 <212> DNA  
 <213> Homo sapiens

<400> 70  
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 agatcctaac agagtgcac ttgtgctttt cctaacagac ctgtcggact ggctttttct 180  
 cttttaaggga tatagagaaa gcaaaattag caaatctagt ttcttgtcac ttacttagga 240  
 gggaggaaaa gagagaaa atgcacttgg gaatgggagg ccttgctttt aatttaccag 300  
 atgccagtta gagcgtaaat gccacacgag ccagagagggt caccctgctg agcatggctt 360  
 gactgttgca gcctctttct gcgactccag acatgcgatg tctgttagct gattctagcc 420  
 ttcagatgca gcccgagat gtaaccctga ggctggagtc ctgtggctct aatccagac 480  
 agaggcaact ccaccaagtt ctggtttggg tcagaaatag agggaaagga tgaatgaaag 540  
 aagatacaaa gaaataatga acaagtgaat tctttcagct gcttacttgg gtggtctgca 600  
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 gctcagaggg gttgtgtggg aagtgaaga aggggt 696

<210> 71  
 <211> 1207  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> unsure  
 <222> (957)  
 <223> a, c, g or t

<400> 71  
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 gccaggcct gacccctaa ggcaggcact gctcogtgat ccaggaaacca cctctctcta 120  
 cagctgggag tgagcagtc gagagggaga cagccttgcc cgggtgctacc cagcaagcta 180  
 gtcaccgagt gggcagaggg aggagcggcc ctaccggat gtcaagcagc ctgggtcccc 240  
 agtccagctc tgcctgtccc tcgcaataac gcctcagtga cgaccatttg tgagccatct 300  
 ctctgtctca ggcacggtgc tacatgccaa cgaaacctgc tccattgaa ccctggccag 360  
 ccagtgaaga aagggttggg cctgggaggt gccactttac agacaggggc accaaggggc 420  
 aggggtggcag gagggcccacc ggacgttccc catgaagtag cagtcccagc atccacaccc 480  
 agcaggcacc acgtggccc gcagcctccc tgccagcacg cctggcttcc cggcctcgga 540  
 acttgatctg ctccctcttc cggacaactgg ggctcctgcc aagtcctggg ctgggcagca 600  
 actgctgaac attctaagaa atccctccca gggttttctc aggagcccgg gtggggcagg 660  
 aagtcgccag gggctgaggg gaccgtggcg gcagggtggca cccagagcag cactctcctg 720  
 gggcccaggc tgttgggcca gaggcaggac tgtgaggcct agtgtagggc ctcttgcag 780  
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 ccaggggctg ggctgtccc agctctggtc ccccgcccc aggtcctgga cgctggntcc 960  
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 agatgagctc gcgcgggac gacctgctgc cgagtcctgc cggacaggca caggcaggga 1080  
 gtgaaaatta tctaccctt tttatttctt aataactgaa tgaaaataaa cattgggtgg 1140  
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 gttatgc 1207

<210> 72  
 <211> 263  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> unsure  
 <222> (231)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (239)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (242)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (248)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (259)  
 <223> a, c, g or t

<400> 72  
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 cattttactt tacctgtcta cagtgttttg cgcaattgac cactccttcc tttttgaagt 120  
 attttctttc cttggtttct gaaatactgt tatcttcccta tctcactggc catacattct 180  
 agtctccttt gctagtttat tatggttttc atcttctcaa caacaatttt ntttttttng 240  
 gnggagangg agtcttgcn tgt 263

<210> 73  
 <211> 579  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> unsure  
 <222> (547)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (555)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (558)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (564)  
 <223> a, c, g or t

<220>

<221> unsure  
 <222> (575)  
 <223> a, c, g or t

<400> 73  
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 ctcttagtaa ttagttttgt ttgtttttgt ttgtttttaa tgttgtgtgt atcttaaggt 120  
 gtaaattgcag acaaagttgg aattgaagct gccgaaatgc tattagcaaa tcttagacat 180  
 ggtggtactg tggatgagta tctgcaagac caggtaatga cacatttagg ttaaaaaccc 240  
 tctaacctgt tagatttgaa tatgtggtag attgaatata aatttaaata attgactttc 300  
 agacactaat tagcaagtcc tacttcaata atttaaaaaa atattctggg attgaccact 360  
 ctcaaatttc agccctcatt ttactttacc tgtctacagt gttttgcgca attgaccact 420  
 ccttcctttt tgaagtattt tctttccttg gtttctgaaa tactgttata ttctatatct 480  
 actggccata cattctagtc tcttttgcta gtttattatg gttttcatct tctcaacaac 540  
 aattttnttt ttttngngng aganggagtc ttgcnatgt 579

<210> 74  
 <211> 339  
 <212> DNA  
 <213> Homo sapiens

<400> 74  
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 actttccatt gtgtataaga taacgataat catagaatta atattattca acttccttgt 180  
 gtcttttgca catttctgta cagtctgtt ttgtttgtt actgtcattc tcaaagtact 240  
 caagttgaat ttgtcactt tggatttctt ccaggaatat gtgagagaca tttaggtctc 300  
 taatgatgaa gtattttcta ggcgtaatgc aaaagattg 339

<210> 75  
 <211> 299  
 <212> DNA  
 <213> Homo sapiens

<400> 75  
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 ggtgacttcg ttccacctc cccttatata ttgttcttcc ttctctctta aattctctaa 120  
 atctctgctt atacagagca atctggctct ctctggcctc tccagtcata atacatcata 180  
 ctcacattca ccatcttgag aagtgcagta agccacataa atgcagcaga agtaacctat 240  
 gcagtcctag gaggctgtgg ttttgagttg cttttttttt tcttttggga gacggagcc 299

<210> 76  
 <211> 247  
 <212> DNA  
 <213> Homo sapiens

<400> 76  
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 gaaatggagg ctgagaggga tatgtagtag ctaaatgtta gagctaggat tganacccaa 180  
 attgacttct gagtatagat ttcccccaa ctgtatgata cttcatattt ggagtcagct 240  
 tgaagta 247

<210> 77  
 <211> 254

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 77

```

tgtatatatttg agctcctact gtgtggcaag gcctatggta agcattttat tttggtaact 60
tgtttaaatcc tcattacaat tctgtggtaa atgctattat ctgtttttat attgaaggga 120
tgaaatggag gctcagaggg atatgtagta gctaaatggt agagctagga ttgaaaccca 180
aattgacttc tgagtataga tttcccccca actgtatgat acttcatatt tggagtcagc 240
ttgaagtaat tcac 254

```

&lt;210&gt; 78

&lt;211&gt; 504

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 78

```

tgatttgatt tggtttttaa atagaatagt tgtactctga gggaggaggg aaatgcttaa 60
acaatactaa gaattccatt ctttagagac aaattactta gaagttgata gtgacatatt 120
gaaaggggtg ttgattggtg gattattcag gtgatgaaga tgatggtagg ggccatggcg 180
gctgagggag aatgagtcct aaacactgag gaggcacaaa agattgggtg gctggatata 240
ataggaaact ggaacgaaag aaggagaaga gaatggcgat actgataaaa aatagaatga 300
aagaagatgt gtggaaaaga aagtttcact ttgaaggctt gatttttgaa gtgatggcag 360
atatagatat acatccaata gatgagtggg aaaagtaaat caaacagaaa tgaaaaattg 420
agtccaagat tgatgggaga ctaataatgg ggaggactga gcctgggggc aactacatta 480
gtaacagtgg caggttttgt tttt 504

```

&lt;210&gt; 79

&lt;211&gt; 210

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (80)..(99)

&lt;223&gt; a, c, g or t

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (173)

&lt;223&gt; a, c, g or t

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (175)

&lt;223&gt; a, c, g or t

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (206)

&lt;223&gt; a, c, g or t

&lt;400&gt; 79

```

gtccctctag agaaccctga ctaatacagg tggttcctgg ctcatggcag tgtgactcca 60
gtctttacat ggcgttcccn nnnnnnnnnn nnnnnnnnnc aaatttcctc ttttcataag 120
gaccgtggta ttggataggg gtccacccta cttcgatatg accttatttt aantncatct 180
ttgatgacct tgtttccacg taaggnaca 210

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<210> 80  
 <211> 161  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> unsure  
 <222> (116)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (148)  
 <223> a, c, g or t

<400> 80  
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 gaagggtggaa aggggacctg agtccaggca tgtgggcagc ctggagaagg cgaganaatg 120  
 gattcttccc cagaatccct ggaaaggnac gtggccctaa c 161

<210> 81  
 <211> 112  
 <212> DNA  
 <213> Homo sapiens

<400> 81  
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 atatctcatt gcatactttt atgtaacttt gtcttagaaa aacaagagtt ct 112

<210> 82  
 <211> 277  
 <212> DNA  
 <213> Homo sapiens

<400> 82  
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 aaaatataat tctaaaaata ttcaagtagc caattggaag gtggaaaaaa gaaaaagaac 180  
 aaaaaataga acagcactaa acaaaaaata aaatcgaga cctaggccct gacatatcaa 240  
 taattatatt aacatgtaaa tggctctaat tttaacca 277

<210> 83  
 <211> 637  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> unsure  
 <222> (92)..(196)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (230)..(316)  
 <223> a, c, g or t

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (367)..(428)

&lt;223&gt; a, c, g or t

&lt;400&gt; 83

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gtccttnggt gttgcaccaa acaggctaag aagcaatgac attgattatg aggaacttgg 60
aactcagatg tattaatttc ctattgtgtc tnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 120
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gtaatgtggc cattatcttg cctacaacct cagaggatgt tgataatgta aagggtagtg 540
aattggggag ttcatagggt ttgatagttg acaaatacag agtgtagtat taggtagggg 600
ttttttggca ggggtgcagt gcccatacct gtaatgt 637

```

&lt;210&gt; 84

&lt;211&gt; 577

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 84

```

caggcataag ccaccacacc tagccaagaa accattcttt gaacacaagc aaatatactt 60
tggagaaaaa tttaataatc ctggcagggc tacattcaac ataattctgt tatgggggaa 120
ggcagcatgc tttggctgct cagtgaagta tggtctgtac aaccaagtga aattgctaaa 180
aaaagattct cctgtataca gtaacttaaa gtgatgcagt ctacttaaga tcagatctga 240
gttacaaaat caaaagtgc agctcctatg ttcttttaaa gtccaatctc tttttttcat 300
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gaatttcttg aacgaatctg ttatgaaaag atctactttg ctcatctctg tgccccaata 420
gcaggagctt gaggagaagg agaaaatatt gggtcagagc ttttgattaa tatgtatgat 480
tctattaaac ggggttcacta aaccaaaaaa ggcaaggaaa acagttaaac caagagtctt 540
gaggttcaag tcttgtgatg attaaatcat catccta 577

```

&lt;210&gt; 85

&lt;211&gt; 687

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 85

```

ttcccatggt agccagggtt ggtcttgac tctgaacctc agggtatcac ccccccttgg 60
gctcaggggt catggctgat attacaggca taagccacca cacctagcca agaaaccatt 120
ctttgaacac aagcaaatat actttggaga aaaatttaat aatcctggca gggctacatt 180
caacataatt ctgttatggg ggaaggcagc atgctttggc tgctcagtga gctatgttct 240
gtacaaccaa gtgaaattgc taaaaaaga ttctcctgta tacagtaact taaagtgatg 300
cagtctactt aagatcagat ctgagttaca aaatcaaaag tgacagctcc tatgttcttt 360
taaagtccaa tctctttttt tcattgttgt gctccaaatg ccttgagtac ctgatgtaga 420
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gagcttttga ttaatatgta tgattctatt aaacgggttc actaaaccaa aaaaggcaaa 600
ggaaaacagt taaaccaaga gttcttgagg ttaaagtctt gtgatgatta aaatcatcat 660
cctaagatga tgatgacata aactttc 687

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&lt;210&gt; 86

35

<211> 77  
 <212> DNA  
 <213> Homo sapiens

<400> 86  
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 cttgcatgca catcccc 77

<210> 87  
 <211> 575  
 <212> DNA  
 <213> Homo sapiens

<400> 87  
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 ttgtctgact ttccaagctt tattaggcat caaacaaaac tgaagtgcct ttttaagattc 180  
 aagtctccta cgtcgtctaa ggcagagtaa gtagccttca gtactatatt ttactctaata 240  
 ttttttttaa cacaatggca gtactataag tatgaaactt tgggtataaat gtcagattct 300  
 agattgtgct cctgctttct gcacactcta atatttttaa acatctcgaa aatacagagt 360  
 ggcagcaaaa ttacctgtaa aaacatacta gctcaagagt ttgacaggct caaaataaat 420  
 taccttaaat acattaaaca agaagtgtat ttgttatata gtatgtactg accaaaatta 480  
 aagtgcaggt tgtacagaaa gagctgcttg tgttatttta tgagcaaaat gaaaagctaa 540  
 tttggtacat ttaaaaatca gcatctagca aattc 575

<210> 88  
 <211> 663  
 <212> DNA  
 <213> Homo sapiens

<400> 88  
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 gccccaccct actcccagca tatgcacaca cacacgtgca cacacaatac tcacttaaca 120  
 aacattttaat ttattgaaca tttattatat gccaaagctg gtataagaca ccaaagagt 180  
 aagacagaaa gtattcttcc ctggagcttt gtctgacttt ccaagcttta ttaggcatca 240  
 aacaaaactg aagtgccttt taagattcaa gtctcctacg tcgtctaagg cagagtaagt 300  
 agccttcagt actataattt actctaattt ttttttaaca caatggcagt actataagta 360  
 tgaaactttg gtataaatgt cagattctag attgtgctcc tgctttctgc acactctaata 420  
 attttttaaac atctcgaaaa tacagagtgg cagcaaaatt acctgtaaaa acatactagc 480  
 tcaagagttt gacaggctca aaataaatta ccttaaatat attaaacaag aagtgtattt 540  
 gttatacagt atgtactgac caaaattaaa gtgcagggtg tacagaaaga gctgcttggtg 600  
 ttattttatg agcaaaatga aaagctaatt tggtagattt aaaaatcagc atctagcaaa 660  
 ttc 663

<210> 89  
 <211> 80  
 <212> DNA  
 <213> Homo sapiens

<400> 89  
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 ttaaaaatac tgtatactaa 80

<210> 90  
 <211> 496



&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 90

```

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ccaggatggg cgtgatctcc tgacctcgtg atccgctgcc ttggctctcc aaagtgctgg 120
aattacaagc gtgacgcact gtgcccagct tagtatacag tatttttaac aagaattata 180
gtaaaatatt atttgaatag aggcttggtt tctgaaacac catccaatct gaaagtagaa 240
gaaaaaggct ggggtgtggtg gctcatgcct gtaacccag cactttggga agctgaggcg 300
ggcggatccc ttgagctcag tttgagacca ggctgggcaa ctccatcttt accaaaaaat 360
acaaaaatga gccaggcatg gtgggtgtaca cctgtggtcc cagcggctct gggggctgag 420
gtggggaggaa ggcttggggc taggaggtgg aggttgagct gagccaggat tgtgccactg 480
ccgatagagc cagata                                     496

```

&lt;210&gt; 91

&lt;211&gt; 385

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 91

```

gaaatgggtc cggacagggt aaaacaaaaa tccaatactg ccgtagtttc taggtggata 60
taacattttt agaaatctta taatacaata ttaacttcat tggctgaacc caagcctttc 120
agcctttata gatttgccat gatcctaata catataagca ttcattgtat tcattattaa 180
ttacttcata gattcagtg gtgacgaagg gagatgattt ttaacaaata ataaagttaa 240
atgatctagt tttgctatgt tgnttgagca acatcaaata gttttgctaa aatagataat 300
ttatagtgat ttttttttca ctatggnatt ttcttaaata tattaagggc tttcattttc 360
tgataccacc tagtttaatt gggggg                                     385

```

&lt;210&gt; 92

&lt;211&gt; 500

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 92

```

gaaatgggtc cggacagggt aaaacaaaaa tccaatactg ccgtagtttc taggtggata 60
taacattttt agaaatctta taatacaata ttaacttcat tggctgaacc caagcctttc 120
agcctttata gatttgccat gatcctaata catataagca ttcattgtat tcattattaa 180
ttacttcata gattcagtg gtgacgaagg gagatgattt ttaacaaata ataaagttaa 240
atgatctagt tttgctatgt tgtttgagca acatcaaata gttttgctta aaatagataa 300
tttatagtga tttttttttc actatggtat tttcttaaata atattaagtg cttttcattt 360
tctgatacca cctagtttaa ttgggggtga atatcagaga aattagaatg ttatttcagc 420
tgaaggagta cagttttttt tttctcttct tagagaatat agtgcctcag atacagtcca 480
caacaaaaat tttggtttag                                     500

```

&lt;210&gt; 93

&lt;211&gt; 364

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (19)

&lt;223&gt; a, c, g or t

&lt;220&gt;

&lt;221&gt; unsure

<222> (21)  
 <223> a, c, g or t

<220>  
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 <223> a, c, g or t

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 <222> (40)  
 <223> a, c, g or t

<220>  
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 <222> (60)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (70)  
 <223> a, c, g or t

<220>  
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 <222> (92)..(93)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (95)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (97)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (121)..(122)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (131)  
 <223> a, c, g or t

<220>  
 <221> unsure  
 <222> (148)  
 <223> a, c, g or t

<400> 93  
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 nnatattaat nagatgtatc aaactgtnac aatatgtgaa gagtattgtg tatatacaaa 180  
 caggaaacaa ttgaaagcct tcaacatgtg tgggtggggg gagagataac tgaattaaca 240  
 ggccatgtag taaaacttaa aatcaaatcc agtagtcttg aaggatatag aattgttttag 300

ttttgaagggt atagtaatta agtactgcgc actaaaaaaaa actgaccaaaggccggggtg 360  
cggt 364

<210> 94

<211> 1646

<212> DNA

<213> Homo sapiens

<400> 94

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nnatattaat	nagatgtatc	aaactgtnac	aatatgtgaa	gagtattgtg	tatatacaaa	180
caggaaacaa	ttgaaagcct	tcaacatgtg	tgggtggggg	gagagataac	tgaattaaca	240
ggccatgtag	taaaacttaa	aatcaaattc	agtagtcttg	aaggtatagt	aattgttttag	300
ttttgaagggt	atagtaatta	agtactgcgc	actaaaaaaaa	actgaccaa	aggccggggtg	360
cggtgggtca	cgcctgtaat	cccagcactt	tgggaggccg	aggcggggcg	atcacctgag	420
gtcaggaggt	cgagaccagc	ctggccaaca	tggtgaaacc	ccgtctctac	taaaaacaca	480
aaaattagcc	gggcatgggtg	gctcacgcct	gtaatcccag	cactctggga	ggccaagaac	540
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aagggtctagc	aaaagatgct	tgggaaatcc	ctcgagaatc	tttgcgacta	gagggttaaac	660
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caatcaaaac	actaaaacca	ggtacaatga	tgccagaagc	tttccttcaa	gaagctcaga	780
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caatttacat	tgtcactgaa	tttatgtcaa	aagggtgctta	ttccctttct	attcgtgatt	900
gggatgagat	aaggggtgac	aatgtgaaac	actacaaaat	taggaaactt	gacaatgggtg	960
gatacatat	cacaaccaga	gcacaatttg	atactctgca	gaaattgggtg	aaacactaca	1020
cagaacatgc	tgatggttta	tgccacaagt	tgacaactgt	gtgtccaact	gtgaaacctc	1080
agactcaagg	tctagcaaaa	gatgcttggg	aaatccctcg	agaatctttg	cgactagagg	1140
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aagtagcaat	caaaacacta	aaaccaggta	caatgatgcc	agaagctttc	cttcaagaag	1260
ctcagataat	gaaaaaatta	agacatgata	aacttgttcc	actatatgct	gttgttttctg	1320
aagaaccaat	ttacattgtc	actgaattta	tgtcaaaaga	gctccacaga	cgctgcacag	1380
ctgtgaactc	aactccagcc	ctcagggaag	gcagctcgca	aagacctagg	gcagcccgcc	1440
gatttcccaa	aaagagcact	gagaaccag	caagcgtttt	tctatatgat	gctggcgcca	1500
gccagtgag	ggaagtgcaa	caagtatgg	tctcctgctt	gtcaactgtg	gaaacagcga	1560
ccctgaaagt	ggaggagcca	cagctggaat	cgtgttcgtc	tgaatacacg	cacctttcct	1620
atgagccttg	taaagccagt	cgttga				1646

<210> 95

<211> 415

<212> DNA

<213> Homo sapiens

<220>

<221> unsure

<222> (109)..(170)

<223> a, c, g or t

<220>

<221> unsure

<222> (323)

<223> a, c, g or t

<220>

<221> unsure

<222> (325)

<223> a, c, g or t

&lt;400&gt; 95

```

gagagtgttt tagcccagaa tagtgggaacc ctcaggcaaa gtttataaaa attaatacca 60
ctgcagaaag tgattaaata ccatggactg taggtttagg tttctgctnn nnnnnnnnnn 120
nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnngtgcata 180
atggagattt taatagtagc catctcataa ggtggttgca aagggttaaat gtgttaatat 240
gcatgatgca catagaacaa tgcctagcac atagtagaga tacataatca ctactatata 300
ctggtaccag tananggtca ggtcttatgg acctaaggctc atataactta gtctcttcca 360
agattcttga aatgatttct caaaacaaga gaataataag aagaaacggt atgaa 415

```

&lt;210&gt; 96

&lt;211&gt; 504

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (212) .. (231)

&lt;223&gt; a, c, g or t

&lt;400&gt; 96

```

atataccta atgagcatgc ccattattcc tctgtcctat gaaacaaaaa tggcattttt 60
caatggattt gttttggata tataattagt tcatttgctg tttagaagcc ttgccaaaag 120
tgttttagatt ttggtactgc aactgctttc ctcttgccca gaaatgtttt gcctcttctt 180
ttcctacaag ttaaagtgtc taaatataaa gnnnnnnnnn nnnnnnnnnn naattccta 240
gtgaaaggca ctagctgtct aatagggttc atgtatcatt actattacta tatgtatctt 300
aatgtagtct atgtagggtt ttatcagaaa gtgtaccttt ctatgggtta ttattttata 360
ttctggggcc ttttatctca gatataaacc atgaacagta atgatatgcc ctgacatata 420
aatcttagta aaaagtgatt aaaaatctaa aactcagtat gaaaaacata tcttggttagc 480
ataaattaaa accttttatt gttt 504

```

&lt;210&gt; 97

&lt;211&gt; 516

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 97

```

atataccta atgagcatgc ccattattcc tctgtcctat gaaacaaaaa tggcattttt 60
caatggattt gttttggata tataattagt tcatttgctg tttagaagcc ttgccaaaag 120
tgttttagatt ttggtactgc aactgctttc ctcttgccca gaaatgtttt gcctcttctt 180
ttcctacaag ttaaagtgtc taaatataaa ggggtatgtg tgtgtgtgtg taattccta 240
gtgaaaggca ctagctgtct aatagggttc atgtatcatt actattacta tatgtatctt 300
aatgtagtct atgtagggtt ttatcagaaa gtgtaccttt ctatgggtta ttattttata 360
ttctggggcc ttttatctca gatataaacc atgaacagta atgatatgcc ctgacatata 420
aatcttagta aaaagtgatt aaaaatctaa aactcagtat gaaaaacata tcttggttagc 480
ataaattaaa accttttatt gtttaaaaaa aaaaaa 516

```

&lt;210&gt; 98

&lt;211&gt; 400

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 98

```

aattagatct ttcctgcaat aaggatctag gtggagggtt tgaagactcg ccggctcagt 60
tggtcatgct aaagcatcta caagtcctag atcttcacca gtgctcacta acagcagatg 120
acgtgatgtc actgaccag gtcattcctt tactttcaaa tcttcaagaa ttggatttat 180

```

40

cagccaacaa	aaagatgggc	agttcttctg	aaaacttact	cagcaggctc	cgattttttac	240
cagcattgaa	gtcattagtt	atcaacaact	gtgcttttga	gagtggagact	tttacagctc	300
ttgctgaagc	ctctgttcac	ctctctgctc	tggaaagtatt	caacctttct	tgggaacaag	360
tgtgttgggtg	ggcaacttga	agctgcttct	gggaaacact			400

&lt;210&gt; 99

&lt;211&gt; 2352

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 99

atthttgattg	aacacaggct	tgacagaatc	ttctttttctt	cttagaaatc	ctagaaaaca	60
gaaagcaaca	ggaagatgtc	ttattgggaa	ctacccccat	caacttcacc	atgagtcaaa	120
caaggaagaa	aacttctca	gaaggagaaa	ctaagcccca	gacttcaact	gtcaacaaat	180
ttctcagggg	ctccaatgct	gaaagcagaa	aagaggacaa	tgaccttaaa	acaagtgatt	240
cccaacccag	cgactggata	cagaagacag	ccacctcaga	gactgctaag	cctctcagtt	300
cagaaatgga	atggagatcc	agtatggaga	aaaatgagca	tttctgcag	aagctgggca	360
aaaaggctgt	caacaagtgt	ctagatttga	ataactgtgg	attaacaaca	gcgggacatga	420
aagaaatggg	agaagcattt	gagatgattc	ctgaacttga	agagctaaat	ttgtcttggg	480
acagtaaagt	gggaggaaat	ttgcctctga	tccttcagaa	gttccaaaaa	gggagcaaga	540
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aactgctacc	tatgctgcaa	agtctcgaag	tacttgatct	ttccattaac	agagacattg	660
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aagactcgcc	ggctcagttg	gtcatgctaa	agcatctaca	agtcctagat	cttcaccagt	900
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acctttcttg	gaacaagtgt	gttgggtggc	acttgaagct	gcttctggaa	acactaaagc	1200
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tcaaagagct	aatcgagctg	gatattagcc	ttcgaccatc	aaatthttcg	gattgtggac	1440
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tgaaaagatg	gattctccca	gcttcacagg	aggaagaact	agaatgcttt	gaccaagata	1560
aaaaaagaag	cattcacttt	gacctgggtg	ggtttcagta	aactgatttc	ccatgtccta	1620
ctaagctaca	aaccattctc	caaaggaaaa	gaacatgaac	gaattccaga	gtcatgaact	1680
gaatttcaac	ttctgggcca	tttaattggg	cttatattac	aagagctttg	taaatatata	1740
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aatacacata	tatatgtaaa	tatatatata	atatctaata	tgagcatgcc	attattctct	1860
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ttgctgttta	gaagccttgc	caaaagtgtt	tagatttttg	tactgcaact	gctttcctct	1980
tgcccagaaa	tgthttgcct	cttctthttcc	tacaagttaa	atgttctaaa	tataaagggg	2040
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tcattactat	tactatatgt	atcttaattgt	agtctatgta	ggthttttatc	agaaagtgtg	2160
cctthttctatg	gtttattatt	ttatatctctg	gtgcctthtt	tctcagatat	aaacctatga	2220
cagtaatgat	agtcactgac	atataaatct	tagtaaaaaag	tgattaaaaa	tctaaaactc	2280
agtatgaaaa	acatatcttg	ttagaataaa	ttaaaacctt	ttattgttta	aaaaattgtt	2340
aaaaaaaaaa	aa					2352

&lt;210&gt; 100

&lt;211&gt; 565

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

41

```

<400> 100
attttgattg aacacaggct tgacagaatc ttcttttctt cttagaaatc ctagaaaaca 60
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caaggaagaa aacttcctca gaaggagaaa ctaagcccca gacttcaact gtcaacaaat 180
ttctcagggg ctccaatgct gaaagcagaa aagaggacaa tgaccttaaa acaagtgatt 240
cccaacccag cgactggata cagaagacag ccacctcaga gactgctaag cctctcagtt 300
cagaaatgga atggagatcc agtatggaga aaaatgagca tttcctgcag aagctgggca 360
aaaaggctgt caacaagtgt ctagatttga ataactgtgg attaacaaca gcggacatga 420
aagaaatggt tgccttgctg ccttttctcc cagacttgga agaactggat atctcctgga 480
atggtttgta ggtggaacc tcctttccat cactcagcaa atgcatctgg tcagcaagtt 540
aaaaatcttg aggctgggta gctgc
565

```

```

<210> 101
<211> 13
<212> PRT
<213> Homo sapiens

```

```

<400> 101
Met Leu Leu His Asp Ile Asp Trp His Leu Met Ser Ile
  1                      5                      10

```

```

<210> 102
<211> 14
<212> PRT
<213> Homo sapiens

```

```

<400> 102
Met Val Leu Pro Gly Ser Leu Ser Met Leu Thr Tyr Gly Met
  1                      5                      10

```

```

<210> 103
<211> 23
<212> PRT
<213> Homo sapiens

```

```

<400> 103
Met Gln Val Leu Tyr Trp Thr Tyr Leu Leu Leu Ile Leu Phe Pro Thr
  1                      5                      10                      15

```

```

Phe Thr Cys Leu Phe Ile Phe
                20

```

```

<210> 104
<211> 26
<212> PRT
<213> Homo sapiens

```

```

<400> 104
Met Asn Leu Tyr Met Asn Leu Pro Ser Ala Val Arg Phe Ser Arg Ala
  1                      5                      10                      15

```

```

Thr Pro Leu Ile Ser Leu Phe Leu Ala Leu
                20                      25

```

42

<210> 105  
 <211> 49  
 <212> PRT  
 <213> Homo sapiens

<400> 105  
 Met Thr Thr Lys Lys Gln Glu Glu Cys Glu Ser Leu Lys Asp Lys Gln  
           1                  5                  10                  15  
 Lys Ala Thr Lys Gln Ser Ile Ser Phe Cys Ile Tyr Ile Ile Lys Val  
                   20                  25                  30  
 Lys Phe Ser Thr Leu Ala Thr Asp Tyr Lys Ser Val Pro Ser Gly Cys  
           35                  40                  45

Cys

<210> 106  
 <211> 61  
 <212> PRT  
 <213> Homo sapiens

<400> 106  
 Met Pro Ser Pro Ser Ala Pro Ser Ile Val Pro Val Leu His Gly Cys  
           1                  5                  10                  15  
 Trp Val His Ile Cys Gln Ala Asp Val Tyr His Thr Leu Leu Lys Gly  
                   20                  25                  30  
 Phe Lys Ser Val Phe Glu Thr Glu Ser His Val Val Ser Pro Arg Leu  
           35                  40                  45  
 Glu Cys Asn Gln Ser Lys Thr Pro Leu Lys Lys Asn Lys  
           50                  55                  60

<210> 107  
 <211> 34  
 <212> PRT  
 <213> Homo sapiens

<400> 107  
 Met Glu Leu Val Met Glu Trp Lys Leu Thr Ile Cys Ser Pro Lys Cys  
           1                  5                  10                  15  
 Ala Thr Thr Thr Gln Gly Leu Gln Thr Asp Ser Tyr Leu Asp Val Val  
                   20                  25                  30

Glu Ser

<210> 108  
 <211> 77  
 <212> PRT  
 <213> Homo sapiens

43

&lt;400&gt; 108

Met Val Asn Pro Ala Gln Glu Met Thr Leu Ser Arg Asn Thr Cys Lys  
 1 5 10 15

Tyr Lys Lys Gln Asp Ile Leu Pro Gln Leu Arg Ser Asp Lys Ile Thr  
 20 25 30

Leu Gly Lys Leu Gln Gly Gln Cys Ala Ser Lys Thr Lys Ser Leu Val  
 35 40 45

Ser Ser Leu Thr Ser Tyr Leu Pro Ala Phe Ile Ile Ile Ser Leu Ser  
 50 55 60

Val Thr Gln Tyr Leu Val Asn Phe Leu Phe Trp His Thr  
 65 70 75

&lt;210&gt; 109

&lt;211&gt; 59

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 109

Met Gln Cys Lys His Phe Phe Leu Thr Tyr Leu Thr Asp Gln Gly Gly  
 1 5 10 15

Gln Val Ala Leu Leu Ser Ser Phe Pro Pro Cys Gly Asp Ser Gly Ile  
 20 25 30

Gln Ala His Ser Ile Thr Arg Leu Ser His Ile Gly Val Phe His Phe  
 35 40 45

Gly Asp Glu Asp Glu Gly Glu Ser Gly Arg Glu  
 50 55

&lt;210&gt; 110

&lt;211&gt; 91

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 110

Met Asp Val Met Gly Lys Leu Lys Gly Ser Cys Asp Glu Thr Gly Ser  
 1 5 10 15

Glu Asn Ser Asp Gly Asp Leu Ser Lys Val Ile Leu Pro Lys His His  
 20 25 30

Leu Ala Ile Met Ile Pro Pro Asn Leu Ser Gln Phe Val Tyr Phe Ile  
 35 40 45

Ser Arg Gly Ser Phe Ser Val Leu Ala Ser Cys Val Phe Val Phe Phe  
 50 55 60

Phe Phe Ser Val Ile Leu Gln Ala Gln Asp Phe Leu Leu Asp Thr Gly  
 65 70 75 80

Arg Ile Ser Leu Leu Lys Glu Ala Gly Gly Thr  
 85 90



```
<210> 111
<211> 45
<212> PRT
<213> Homo sapiens
```

```

<400> 111
Met Gly His Val Asp Gln Leu Ser Pro Arg Thr Thr Asn Leu Ala Cys
  1                               5                10                15
Ser Asp Asp Leu Cys Ser Arg Gln Gly Phe Arg Leu Asp Cys Cys Ser
      20                               25                30
Ser Leu Trp Arg His Asn Pro Asn Cys Glu Leu Leu Asn
      35                               40                45

```

```
<210> 112
<211> 64
<212> PRT
<213> Homo sapiens
```

<400> 112																
Met	Leu	Lys	Met	Ile	Leu	Ala	Ser	Ile	Val	Ile	Asn	Ser	Val	Ile	Pro	
1				5					10					15		
Glu	Phe	Phe	Val	Ser	Pro	Arg	His	Thr	Asn	Phe	Cys	Pro	Leu	Leu	Leu	
			20					25					30			
Phe	Ser	Gln	Ser	Phe	Leu	Leu	Ala	Phe	Leu	Ser	Asn	Arg	Val	Leu	Leu	
		35					40					45				
Thr	Pro	Tyr	Ile	Pro	Phe	Trp	Leu	Val	Arg	Val	Ser	Phe	Ser	Ser	Ser	
	50					55					60					

```
<210> 113
<211> 25
<212> PRT
<213> Homo sapiens
```

<220>  
<221> UNSURE  
<222> (14)

<220>  
<221> UNSURE  
<222> (17)

```

<400> 113
Met Leu Leu Phe Thr Lys Leu Leu Ile Ile Met Val Ile Xaa Ile Asn
  1                      5                      10                      15
Xaa Asn Asn Lys Leu Leu Gln Leu Phe
          20                      25

```

45

&lt;210&gt; 114

&lt;211&gt; 57

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 114

Met Arg Ile Gln Asn Leu Thr Cys Leu Leu Leu Gly Ser Lys Glu Met  
 1 5 10 15

Ser Thr Ser Ser Pro Leu Thr Pro Asn Gly Val Glu Gly Phe Gly Pro  
 20 25 30

Gln His Cys Val Thr Tyr Ser His His Asp Phe Leu Ala Gln Val Thr  
 35 40 45

Pro Ser Val Lys Trp Lys Arg Glu Glu  
 50 55

&lt;210&gt; 115

&lt;211&gt; 147

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 115

Met Asn Glu Ser Trp Ala Gly Pro Gly Pro Ala Glu Arg Ala Glu Glu  
 1 5 10 15

Ala Val Ser Gly Val Gly Val Glu Ala Lys Thr Gln His Ala Gly Gln  
 20 25 30

Gly Ala Gln Pro Gly Gly Met Gly Cys Gly Phe Ser Ser Gly Pro Ile  
 35 40 45

Gly Met Ala Leu Gly Leu Gly Leu Val Gly Thr Ala Ala Thr Arg Gly  
 50 55 60

Gly Ser Ser Ala Trp Pro Asp Ser Thr Cys Asn Val Gly Arg Gln Trp  
 65 70 75 80

Ala Pro Pro Gly Gly Arg Asn Thr Val Arg Ser Met Gln Arg Ala Gly  
 85 90 95

Asp His Gly Ala Cys Asp Leu Arg Ala His Pro Gly Gln Thr Trp Val  
 100 105 110

Arg Gly Gly Leu Gly Arg Gln Asp Ser Glu Gly Leu Gln Gly Val Phe  
 115 120 125

Val Leu Cys Pro Tyr Thr Gly Asp Leu His Gly Arg Val Arg Ser Ile  
 130 135 140

Arg Met Leu  
 145

&lt;210&gt; 116

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<211> 73
<212> PRT
<213> Homo sapiens
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<400> 116
Met Thr Ile Ser Leu Cys Ala Thr Asn Leu Pro Arg Ala Ala Thr Val
  1                      5                      10                      15
Leu Arg Met Lys Pro Lys Leu Pro Gly Ser Gly Pro Val Gln His Glu
          20                      25                      30
Pro His Leu Pro Ser Gln Pro Gln His Pro Leu Leu Phe Phe Gln Ala
          35                      40                      45
Gly Gly Lys Leu Glu Ala His Pro His Phe Thr Gln Thr Leu Gly Ile
          50                      55                      60
Pro Ile Ser Gly Asn Arg Gly Val Phe
          65                      70

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<210> 117
<211> 48
<212> PRT
<213> Homo sapiens
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<220>  
<221> UNSURE  
<222> (46)

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<400> 117
Met Tyr Asn Ile Leu Lys Ala Phe Asp Lys Ile Val His Ile Ile Ser
  1             5             10             15
Asn Thr Ile Leu Tyr Tyr Tyr Gln Gln His Lys Ala Asn Val Ser Lys
      20             25             30
Asn Ser Arg Leu Arg Ile Ser Lys Asn Ser Pro Arg Ala Xaa Phe Arg
      35             40             45

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<210> 118
<211> 38
<212> PRT
<213> Homo sapiens
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<400> 118
Met Leu Pro Val Ser Pro Thr Leu Lys Glu Arg Asn Gln Arg Arg Met
 1             5             10             15
Leu Leu Lys Ser Thr His Leu Ala Ser Val Ser Ser Ala Ser Cys Thr
 20             25             30
Gln Thr Lys His Thr Gly
 35

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47

<210> 119  
 <211> 55  
 <212> PRT  
 <213> Homo sapiens

<400> 119  
 Met Lys Ile Phe Ile Ile Ile Leu Ser Pro Leu Cys Gly Ile Leu Leu  
     1                    5                    10                    15  
 Asn Val Leu Glu Ser Leu Lys Phe Ile Phe Lys Cys Glu Ser Leu Leu  
                     20                    25                    30  
 Phe Val Trp Gly Glu Glu Cys Gln Val Gly Ile Met Asn Gln Ala Leu  
             35                    40                    45  
 Pro Tyr Gln Val Leu Leu Tyr  
             50                    55

<210> 120  
 <211> 92  
 <212> PRT  
 <213> Homo sapiens

<400> 120  
 Glu Ser His Thr Leu Gln Val Ile Leu Gly Cys Glu Met Gln Glu Asp  
     1                    5                    10                    15  
 Asn Ser Thr Glu Gly Tyr Trp Lys Tyr Gly Tyr Asp Gly Gln Asp His  
                     20                    25                    30  
 Leu Glu Phe Cys Pro Asp Thr Leu Asp Trp Arg Ala Ala Glu Pro Arg  
             35                    40                    45  
 Ala Trp Pro Thr Lys Leu Glu Trp Glu Arg His Lys Ile Arg Ala Arg  
             50                    55                    60  
 Gln Asn Arg Ala Tyr Leu Glu Arg Asp Cys Pro Ala Gln Leu Gln Gln  
     65                    70                    75                    80  
 Leu Leu Glu Leu Gly Arg Gly Val Leu Asp Gln Gln  
                     85                    90

<210> 121  
 <211> 85  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> UNSURE  
 <222> (51)..(72)

<400> 121  
 Met Ile Lys Val Ser Leu Thr Ser Ala Pro Lys Val Ser Ser Leu Glu  
     1                    5                    10                    15  
 Gly Thr Asn Arg Arg Glu His Ser Asp Thr Gln Gly Pro Leu Ser Val

48

20 25 30  
 Pro Trp Lys Pro Ser Asp Leu Cys Arg Pro Ile Ser Val Arg Lys Trp  
 35 40 45  
 Val Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 50 55 60  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Arg Thr Thr Gln Ser Ser Trp Gln  
 65 70 75 80  
 Ile Leu Asn Lys Gly  
 85

<210> 122  
 <211> 20  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> UNSURE  
 <222> (15)

<400> 122  
 Met Gly Gly Ala Trp Ser Ile Ala Gly Pro Leu Thr Gly Phe Xaa Phe  
 1 5 10 15

Arg Leu Thr Phe  
 20

<210> 123  
 <211> 103  
 <212> PRT  
 <213> Homo sapiens

<400> 123  
 Phe Tyr Phe Leu Phe Ser Phe Val Leu Arg Trp Ser Phe Thr Leu Val  
 1 5 10 15

Thr Gln Ala Gly Val Gln Trp Cys Asp Leu Gly Ser Leu Gln Pro Pro  
 20 25 30

Pro Pro Arg Leu Lys Ala Phe Ser Cys Leu Gly Leu Pro Ser Ser Trp  
 35 40 45

Asp Tyr Arg His Ala Leu Gln Arg Pro Ala Asn Phe Ala Phe Leu Val  
 50 55 60

Glu Ile Gly Phe His His Val Gly Gln Ala Gly Pro Gln Leu Leu Thr  
 65 70 75 80

Ser Gly Asp Pro Ser Ile Leu Ala Ser Gln Ser Ala Gly Ile Thr Gly  
 85 90 95

Val Thr Ala Val Pro Gly Pro  
 100

49

<210> 124  
 <211> 48  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> UNSURE  
 <222> (13)..(43)

<400> 124  
 Met Val Val Ile Gln Ala Xaa Glu Glu Glu Lys Thr Xaa Xaa Xaa Xaa  
     1                    5                    10                    15  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
                     20                    25                    30  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ile Trp Lys Ile Cys  
                     35                    40                    45

<210> 125  
 <211> 95  
 <212> PRT  
 <213> Homo sapiens

<400> 125  
 Met Ser Ser Tyr Met Ile Asn Lys Phe Leu Pro Ile Lys Lys Val Lys  
     1                    5                    10                    15  
 Ile Pro Gly His Lys Val Phe Ser Thr Asp Ile Met Phe Leu Lys Phe  
                     20                    25                    30  
 Val Ser Ile Ala Thr Leu Leu Arg Arg His Thr Asp Ile Ser Glu Asp  
                     35                    40                    45  
 Leu Arg Val Leu Gln Asn Thr Glu Lys Ile Ser Arg Arg Lys Gly Lys  
     50                    55                    60  
 Gly Glu Thr Lys Lys Leu Lys Glu Gly Leu Thr Tyr Lys Trp Asn Asp  
     65                    70                    75                    80  
 Leu Lys Arg Asn Gly Glu Pro Gly Glu Thr Gly Val Ser Gln Ser  
                     85                    90                    95

<210> 126  
 <211> 48  
 <212> PRT  
 <213> Homo sapiens

<400> 126  
 Met Ile Lys Tyr Phe Lys Ser Asn Asn Tyr Lys Phe Asn Tyr Tyr Lys  
     1                    5                    10                    15  
 Thr Ser Ser Leu Thr Ser Asp Cys Phe Val Leu Ser Phe Lys Ile Ile

50

20

25

30

Met Val Cys Leu Arg Val Cys Leu Leu Asn Thr Phe Ala Tyr Leu Pro  
                   35                                  40                                  45

&lt;210&gt; 127

&lt;211&gt; 98

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 127

Met Glu Phe Arg Ser Val Ala Gln Val Gly Val Gln Trp Arg Asp Leu  
   1                                  5                                  10                                  15

Gly Leu Leu Gln Pro Leu Pro Leu Gln Phe Lys Gln Phe Tyr Cys Leu  
                   20                                  25                                  30

Ser Leu Ser Ser Ser Trp Asp Tyr Arg His Ser Pro Pro His Pro Ala  
                   35                                  40                                  45

Asn Phe Leu Tyr Phe Ala Lys Ile Leu Tyr Ile Ala Lys Arg Phe His  
                   50                                  55                                  60

His Val Gly Gln Ala Gly Leu Ala Leu Leu Thr Ser Gly Asp Pro Pro  
   65                                  70                                  75                                  80

Thr Ser Ala Ser Gln Ser Ala Gly Ile Thr Gly Leu Ser His Cys Ala  
                                   85                                  90                                  95

Gln Pro

&lt;210&gt; 128

&lt;211&gt; 50

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 128

Met Gly Lys Arg Arg Asp Ser Trp Thr Asn Arg Glu Arg Gln Leu Glu  
   1                                  5                                  10                                  15

Asn Lys Ser Met Gln Lys Ile Ile Tyr Asn Lys Ile Met His Leu Thr  
                   20                                  25                                  30

Leu Val Thr Lys Gln Ile Ser Tyr Pro His Phe Ser Leu Ser Val Phe  
                   35                                  40                                  45

Val Ser  
       50

&lt;210&gt; 129

&lt;211&gt; 16

&lt;212&gt; PRT

51

&lt;213&gt; Homo sapiens

&lt;400&gt; 129

Met Leu Leu Phe Val Leu Ser Leu Val Phe Gln Tyr Gln Phe Asn Thr  
 1 5 10 15

&lt;210&gt; 130

&lt;211&gt; 54

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 130

Met Ala Leu His Cys Phe Thr Ser Gly Leu Trp Ile Ala Ser Val Arg  
 1 5 10 15

Lys Lys Val Lys Met Lys Glu Lys Val Glu Gln Ile Leu Ala Thr Glu  
 20 25 30

Pro Pro Glu Asp Ser Cys Pro Phe Ser Asn Lys Leu Ser Gly Lys Cys  
 35 40 45

Cys Cys His Gly Ser Thr  
 50

&lt;210&gt; 131

&lt;211&gt; 41

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 131

Met Cys Ala His Lys Gly Lys Ala Met Arg Glu Arg Thr Gln Pro Glu  
 1 5 10 15

Gly Gly His Leu Ala Ser Gln Gly Glu Ala Leu Arg Glu Thr Lys Pro  
 20 25 30

Ala Arg Leu Gly Thr Val Ala His Gly  
 35 40

&lt;210&gt; 132

&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 132

Met Ala Leu Ile Leu Leu Glu Ala Leu Cys Phe Gly Leu Ile Ile Cys  
 1 5 10 15

Met Asn Arg Glu Ser Ile Ser Thr Leu Ile Phe Tyr Lys His Trp Met  
 20 25 30

Ser Ile Leu  
 35

&lt;210&gt; 133



52

<211> 58  
 <212> PRT  
 <213> Homo sapiens

<400> 133  
 Met Phe Asn Ala Tyr Leu Leu Tyr Asn Asn Gln Val Ile Thr Val Gln  
     1                    5                    10                    15  
 Ile Lys Gly Pro Lys Cys Phe Arg Tyr Asp Ile Ile Leu Ser Ile Val  
                     20                    25                    30  
 Asn Trp Thr Lys Glu Thr Leu Tyr Val Gln Gly Ser Val Glu Gln Pro  
                     35                    40                    45  
 Trp Cys Ser Trp Asp Met Leu Pro Arg Cys  
                     50                    55

<210> 134  
 <211> 27  
 <212> PRT  
 <213> Homo sapiens

<400> 134  
 Met Met Lys Leu Cys Phe Thr Ala Ser Leu Leu His Gly Ala Leu Leu  
     1                    5                    10                    15  
 Trp His Leu Ala Thr Thr Asn Ser Leu Ile Pro  
                     20                    25

<210> 135  
 <211> 46  
 <212> PRT  
 <213> Homo sapiens

<400> 135  
 Met Glu Leu Pro Ser Met Cys Pro Ile Leu Phe Phe Val Thr Val Phe  
     1                    5                    10                    15  
 Phe Met Tyr His Thr Pro Ser Cys Pro Ser Ser Val Pro Gln Thr His  
                     20                    25                    30  
 Gln Ser His Phe Leu Leu Thr Ala Leu Gly Leu Ala Leu Thr  
                     35                    40                    45

<210> 136  
 <211> 77  
 <212> PRT  
 <213> Homo sapiens

<400> 136  
 Met Thr Cys Pro Gly Gly Glu Thr Gly Trp Gly Cys Leu Arg Met Asp  
     1                    5                    10                    15  
 Pro Arg Glu Trp Val Ser Ser Pro Asp Gln Gln Asn Leu Arg Met Cys  
                     20                    25                    30

53

Ala Trp Ile Gln Pro His Leu Lys Leu Gly Leu His Phe Val Ser Gly  
35 40 45

Ala Pro Asn Ala Leu Cys Leu Gly Cys Leu Tyr Ser Trp His Thr Gly  
50 55 60

Glu Ala Leu Ser Pro Ala Gly Pro Gly Cys Cys Cys Ser  
65 70 75

<210> 137

<211> 37

<212> PRT

<213> Homo sapiens

<400> 137

Met Glu Gln Glu Ser Val Pro Ser Met Ser Leu Phe Thr Arg Ile Leu  
1 5 10 15

Ser Gln Pro Ser Leu Phe Pro Trp Gln Ala Leu His Arg Glu Thr Gly  
20 25 30

Lys Arg Ser Thr Val  
35

<210> 138

<211> 59

<212> PRT

<213> Homo sapiens

<400> 138

Met Leu Leu Pro Leu Pro Ala Ile Ser Phe Pro Cys Asn Ser Leu Phe  
1 5 10 15

His Pro Ala Asp Ala Ser Ser Leu Ser Trp Leu Ser Ser Lys Ser Tyr  
20 25 30

Pro Leu Gly Lys Leu Thr Arg Met Leu Gln Ser Asp Gly Val Ser Pro  
35 40 45

Pro Gly Pro Pro Gln Thr Leu Tyr Phe Leu Leu  
50 55

<210> 139

<211> 50

<212> PRT

<213> Homo sapiens

<400> 139

Met Asp Asn Lys Cys Leu Thr Leu Thr Asn Tyr Leu Ala Ile Met Gly  
1 5 10 15

Phe Phe Asp Gln Lys Ser Ser Lys Arg Val Trp Trp Gly Leu Arg Asp  
20 25 30

Pro Ser Ser Leu Pro Lys Asn Met Lys Ser Phe His Phe Gln Tyr Val  
35 40 45

Lys Thr  
50

<210> 140  
<211> 72  
<212> PRT  
<213> Homo sapiens

<400> 140  
Met Arg Val Val Phe Lys Ile Thr Phe Cys Arg Val Val Cys Ser Thr  
1 5 10 15  
Leu Met Leu Lys Gly Ser His Leu Pro Gln Pro Ile Lys Leu Cys Cys  
20 25 30  
Leu Cys Ser Ala Phe Tyr His Lys Asn Met Thr Phe Lys His Lys Asn  
35 40 45  
Thr Leu Tyr Ser Thr Thr Lys Asn Arg Asn Asp Ile Tyr Leu His Cys  
50 55 60  
Phe Pro Ile Ser Leu His Leu Tyr  
65 70

<210> 141  
<211> 863  
<212> PRT  
<213> Homo sapiens

<400> 141  
Met Pro Glu Gln His Lys Asp Pro Arg Val Gln Glu Asn Pro Asp Asp  
1 5 10 15  
Gln Arg Thr Val Pro Glu Val Thr Gly Asp Ala Arg Ser Ala Phe Trp  
20 25 30  
Pro Leu Arg Asp Asn Gly Gly Pro Ser Pro Phe Val Pro Arg Pro Gly  
35 40 45  
Pro Leu Gln Thr Asp Leu His Ala Gln Ser Ser Glu Ile Arg Tyr Asn  
50 55 60  
His Thr Ser Gln Thr Ser Trp Thr Ser Ser Ser Thr Lys Arg Asn Ala  
65 70 75 80  
Ile Ser Ser Ser Tyr Ser Ser Thr Gly Gly Leu Pro Gly Leu Lys Gln  
85 90 95  
Arg Arg Gly Pro Ala Ser Ser Arg Cys Gln Leu Thr Leu Ser Tyr Ser  
100 105 110  
Lys Thr Val Ser Glu Asp Arg Pro Gln Ala Val Ser Ser Gly His Thr  
115 120 125  
Arg Cys Glu Lys Gly Ala Asp Thr Ser Pro Gly Gln Thr Ile Ala Pro  
130 135 140

Thr	Gly	Gly	Ser	Pro	Arg	Ser	His	Asp	Ser	Arg	Pro	Arg	Arg	Arg	Lys	145	150	155	160
Ile	Pro	Leu	Leu	Pro	Arg	Arg	Arg	Gly	Glu	Pro	Leu	Met	Leu	Pro	Pro	165	170	175	
Pro	Leu	Glu	Leu	Gly	Tyr	Arg	Val	Thr	Ala	Glu	Asp	Leu	His	Leu	Glu	180	185	190	
Lys	Glu	Thr	Ala	Phe	Gln	Arg	Ile	Asn	Ser	Ala	Leu	His	Val	Glu	Asp	195	200	205	
Lys	Ala	Ile	Pro	Asp	Cys	Arg	Pro	Ser	Arg	Pro	Ser	His	Thr	Leu	Ser	210	215	220	
Ser	Leu	Ala	Thr	Gly	Ala	Ser	Gly	Gly	Pro	Pro	Val	Ser	Lys	Ala	Pro	225	230	235	240
Thr	Met	Asp	Ala	Gln	Gln	Asp	Arg	Pro	Lys	Ser	Gln	Asp	Cys	Leu	Gly	245	250	255	
Leu	Val	Ala	Pro	Leu	Ala	Ser	Ala	Ala	Glu	Val	Pro	Ala	Thr	Ala	Pro	260	265	270	
Val	Ser	Gly	Lys	Lys	His	Arg	Pro	Pro	Gly	Pro	Leu	Phe	Ser	Ser	Ser	275	280	285	
Asp	Pro	Leu	Pro	Ala	Asn	Ser	Ser	His	Ser	Arg	Asp	Ser	Ala	Gln	Val	290	295	300	
Thr	Ser	Met	Ile	Pro	Ala	Pro	Phe	Thr	Ala	Ala	Ser	Arg	Asp	Ala	Gly	305	310	315	320
Met	Arg	Arg	Thr	Arg	Ser	Ala	Pro	Ala	Ala	Ala	Ala	Ala	Ala	Pro	Pro	325	330	335	
Pro	Ser	Thr	Leu	Asn	Pro	Thr	Ser	Gly	Ser	Leu	Leu	Asn	Ala	Val	Asp	340	345	350	
Gly	Gly	Pro	Ser	His	Phe	Leu	Ala	Ser	Ala	Thr	Ala	Ala	Ala	Arg	Ala	355	360	365	
Gln	Arg	Ser	Glu	Val	Arg	Tyr	Asn	Gln	Arg	Ser	Gln	Thr	Ser	Arg	Thr	370	375	380	
Arg	Ser	Cys	Leu	Lys	Arg	Asn	Ala	Ser	Ser	Ser	Ser	His	Ser	Ser	Thr	385	390	395	400
Glu	Gly	Leu	Gln	Glu	Val	Lys	Arg	Arg	Arg	Gly	Pro	Ala	Ser	Ser	His	405	410	415	
Cys	Gln	Leu	Ala	His	Ser	Ser	Ser	Asn	Thr	Val	Ser	Glu	Asp	Gly	Pro	420	425	430	
Gln	Ala	Val	Ser	Ser	Gly	His	Arg	Cys	Glu	Asn	Lys	Ala	Gly	Thr	Ala	435	440	445	
Pro	Gly	Gln	Thr	Leu	Ala	Pro	Arg	Gly	Gly	Ser	Pro	Arg	Ser	Gln	Ala				

56

450	455	460
Ser Arg Pro His Ile Asn Thr Ala Leu His Val Glu Asp Lys Ala Ile		
465	470	475 480
Ser Asp Cys Arg Pro Ser Arg Pro Ser His Thr Leu Ser Ser Leu Ala		
	485	490 495
Thr Gly Ala Ser Gly Gly Pro Pro Val Ser Lys Ala Pro Thr Met Asp		
	500	505 510
Ala Gln Gln Asp Arg Pro Lys Ser Gln Asp Ser Leu Gly Leu Leu Ala		
	515	520 525
Pro Leu Ala Ser Ala Ala Glu Val Pro Ser Thr Ala Pro Val Ser Gly		
	530	535 540
Lys Lys His Arg Pro Pro Gly Pro Leu Phe Ser Ser Ser Asp Pro Leu		
	545	550 555 560
Pro Ala Thr Ser Tyr His Ser Arg Asp Thr Ala Gln Val Thr Ser Leu		
	565	570 575
Ile Pro Ala Thr Phe Thr Ala Ala Ser Arg Asp Ala Gly Met Arg Arg		
	580	585 590
Thr Arg Ser Ala Pro Ala Ala Ala Thr Ala Ala Pro Pro Pro Ser Thr		
	595	600 605
Leu Asn Asn Thr Ser Gly Ser Leu Leu Asn Ala Val Asp Gly Gly Pro		
	610	615 620
Ser His Phe Leu Ala Ser Ala Thr Ala Ala Ala Arg Ala Gln Arg Ser		
	625	630 635 640
Glu Val Arg Tyr Asn Gln Arg Ser Gln Thr Ser Arg Thr Arg Ser Cys		
	645	650 655
Leu Lys Arg Asn Ala Ser Ser Ser Ser Ser Ser His Ser Ser Thr Glu		
	660	665 670
Gly Leu Gln Glu Val Lys Arg Arg Arg Gly Pro Ala Ser Ser His Cys		
	675	680 685
Gln Leu Ala His Ser Ser Ser Asn Thr Val Ser Glu Asp Gly Pro Gln		
	690	695 700
Ala Val Ser Ser Gly His Arg Cys Glu Asn Lys Ala Gly Thr Ala Pro		
	705	710 715 720
Gly Gln Thr Leu Ala Pro Arg Gly Gly Ser Pro Arg Ser Gln Ala Ser		
	725	730 735
Arg Pro His Ile Asn Ser Ala Leu His Val Glu Asp Lys Ala Ile Ser		
	740	745 750
Asp Cys Arg Pro Ser Arg Pro Ser His Thr Leu Ser Ser Leu Ala Thr		
	755	760 765

57

Gly Ala Ser Gly Gly Pro Pro Val Ser Lys Ala Pro Thr Met Asp Ala  
 770 775 780

Gln Gln Asp Arg Pro Lys Ser Gln Asp Cys Leu Gly Leu Leu Ala Pro  
 785 790 795 800

Leu Ala Ser Ala Ala Glu Val Phe Ser Thr Ala Pro Val Ser Gly Lys  
 805 810 815

Lys His Arg Pro Pro Gly Pro Leu Phe Ser Ser Ser Asp Pro Leu Pro  
 820 825 830

Ala Thr Ser Ser His Ser Gly Asp Ser Ala Gln Asp Thr Ser Leu Ile  
 835 840 845

Pro Ala Pro Phe Thr Pro Ala Ser Arg Asp Ala Gly Ile Arg Arg  
 850 855 860

&lt;210&gt; 142

&lt;211&gt; 29

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 142

Met Ser Tyr Leu Ser Leu Leu Leu Ile Ser Ile Phe Met Val Cys Tyr  
 1 5 10 15

Phe Lys Arg Asn Ser Phe Pro Ile Thr Ile Leu Phe Ser  
 20 25

&lt;210&gt; 143

&lt;211&gt; 32

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 143

Met Pro Trp Pro Met Pro Ile Cys Thr Gly Thr Gln Gly Val Leu Thr  
 1 5 10 15

His Arg Gln Gly Pro Pro Pro Ala Ala Val Gly Val Ser Pro His Thr  
 20 25 30

&lt;210&gt; 144

&lt;211&gt; 29

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 144

Met Asn Ala Phe Leu Leu Glu Arg Met Thr Glu Ser Gln Ala Met Asp  
 1 5 10 15

Ile Gln Thr Cys Ile Phe Gln Thr Leu Leu Glu Asn Lys  
 20 25

<210> 145  
 <211> 48  
 <212> PRT  
 <213> Homo sapiens

<400> 145  
 Met Ile Val Thr Asn Thr Ile Leu Lys Phe Ile His Lys Lys Pro Thr  
           1                  5                  10                  15  
 Thr Ile Thr Pro Thr Lys Gln His Gly Ile Ile Phe Ser Val Pro Glu  
                   20                  25                  30  
 Ala Lys Val Arg Ala Leu Leu Cys Phe Leu Leu Arg Met Pro Ser Pro  
           35                  40                  45

<210> 146  
 <211> 55  
 <212> PRT  
 <213> Homo sapiens

<400> 146  
 Gly Gln Ala Leu Trp Leu Met Pro Val Ile Pro Val Val Ala Lys Ala  
           1                  5                  10                  15  
 Glu Gly Lys Asp His Leu Arg Pro Gly Val Ala Asn Gln Pro Gly Gln  
                   20                  25                  30  
 His Ser Lys Thr Leu Phe Leu Gln Lys Lys Asn Phe Ala Lys Leu Ala  
           35                  40                  45  
 Glu His Gly Gly Ala Cys Leu  
           50                  55

<210> 147  
 <211> 55  
 <212> PRT  
 <213> Homo sapiens

<400> 147  
 Met Ser Arg Phe Arg Ile Gln Thr Ser Glu Thr Ala Pro Ile Pro Leu  
           1                  5                  10                  15  
 Val Ser His Pro His Thr Pro Leu Ser Asn Asn Asn Asn Leu His Leu  
                   20                  25                  30  
 Gly Asn Val Cys Tyr Val Pro Gly His Thr Gly Ile Ile Ser Cys Thr  
           35                  40                  45  
 Pro His Arg His Leu Ile Lys  
           50                  55

59

<210> 148  
 <211> 50  
 <212> PRT  
 <213> Homo sapiens

<400> 148  
 Met Gln Gly Leu His Leu Pro Gln Gly Leu Gly Thr Cys Tyr Ser Ile  
           1                  5                  10                  15  
 Cys Leu Gln Cys Leu Ser Pro His Gly Tyr Phe Phe Val Ala Val Gly  
                   20                  25                  30  
 Leu Ser Ser Asn Val Met Ser Pro Thr Ser Leu Pro Lys Ala Val Leu  
                   35                  40                  45  
 Pro Thr  
           50

<210> 149  
 <211> 31  
 <212> PRT  
 <213> Homo sapiens

<400> 149  
 Met Leu Pro Val Asn Ile Ser His Pro Leu Ser Arg Gly Asn Pro Leu  
           1                  5                  10                  15  
 Leu Ser Ser Lys Phe Ser Lys Phe Phe Leu Ile Glu Phe Ser Gln  
                   20                  25                  30

<210> 150  
 <211> 36  
 <212> PRT  
 <213> Homo sapiens

<400> 150  
 Met Asp Tyr Ser Leu Ser Phe Asp Asn Tyr Thr Trp Gly Phe Gly Glu  
           1                  5                  10                  15  
 Pro Arg Ile Lys Val Gln Ser Phe Asn Asp Leu Leu Ala Pro Gly Leu  
                   20                  25                  30  
 Thr Gln Glu His  
           35

<210> 151  
 <211> 85  
 <212> PRT  
 <213> Homo sapiens

<400> 151  
 Met Ile Arg Ser Lys Gly Thr Asn Phe Gln Ile Leu Ala Glu Leu Phe  
           1                  5                  10                  15  
 Lys Gly Met Asp Phe Leu Trp Leu Gln Leu Ala Arg Leu Phe Gln Lys  
                   20                  25                  30



60

Ala Cys Pro Trp Leu Thr Ala Cys Leu Ala Gln Phe Leu Arg Ser Pro  
           35                          40                          45

Leu Val Met Glu Asn Arg Ala Asp Arg Ile Gln Met Ala Arg Phe His  
           50                          55                          60

Arg Gly Gln Gly Gly Pro Gln Ser Ala Asn Gln Gly Arg Leu Arg Pro  
           65                          70                          75                          80

Glu Lys Gly Ile Ser  
                                   85

<210> 152  
 <211> 73  
 <212> PRT  
 <213> Homo sapiens

<400> 152  
 Met Asp Arg Phe Leu Asn Ser Lys Ala Arg Arg Leu Gly Ser Cys Ser  
       1                          5                          10                          15

His Pro Ala Phe Tyr Leu Leu Cys Val Pro Asp Glu Asp Thr Ser Cys  
           20                          25                          30

Ser Thr Met Tyr Leu Pro Leu Lys Arg Arg Ala Asp Pro Asp Gln Leu  
           35                          40                          45

Phe Ser Asp Leu Leu Gly Gly Thr Gln Arg Leu Trp Arg Leu Trp Pro  
           50                          55                          60

Ser Leu Ala Ser Val Glu Ser Gly Leu  
       65                          70

<210> 153  
 <211> 63  
 <212> PRT  
 <213> Homo sapiens

<400> 153  
 Met Gln Cys Thr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
       1                          5                          10                          15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
           20                          25                          30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Ile Lys Phe Gly  
           35                          40                          45

Met Lys Gln Glu Leu Ser Trp Thr Ile Tyr Asn Leu Leu Arg Tyr  
           50                          55                          60

<210> 154  
 <211> 46  
 <212> PRT  
 <213> Homo sapiens

61

&lt;400&gt; 154

Met Arg Cys Leu Leu Ala Asp Ser Ser Leu Gln Met Gln Pro Gly Asp  
 1 5 10 15

Val Thr Leu Arg Leu Glu Ser Cys Gly Ser Asn Pro Arg Gln Arg Gln  
 20 25 30

Leu His Gln Val Leu Val Trp Val Arg Asn Arg Gly Lys Gly  
 35 40 45

&lt;210&gt; 155

&lt;211&gt; 72

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; UNSURE

&lt;222&gt; (22)

&lt;400&gt; 155

Met Pro Pro Arg Gly Trp Ala Cys Pro Ser Ser Gly Pro Pro Ala Pro  
 1 5 10 15

Gly Pro Gly Arg Trp Xaa Arg Ala Ala Ala Gly Gly Leu Arg Arg Thr  
 20 25 30

Arg Cys Asp Trp Leu Pro Leu Arg Arg Thr Gln Met Ser Leu Arg Arg  
 35 40 45

Ile Asp Leu Leu Pro Ser Pro Ala Gly Gln Ala Gln Ala Gly Ser Glu  
 50 55 60

Asn Tyr Leu Pro Leu Phe Ile Ser  
 65 70

&lt;210&gt; 156

&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; UNSURE

&lt;222&gt; (10)

&lt;220&gt;

&lt;221&gt; UNSURE

&lt;222&gt; (13)..(14)

&lt;220&gt;

&lt;221&gt; UNSURE

&lt;222&gt; (16)

&lt;220&gt;

&lt;221&gt; UNSURE

&lt;222&gt; (20)

62

&lt;400&gt; 156

Met Val Phe Ile Phe Ser Thr Thr Ile Xaa Phe Phe Xaa Xaa Glu Xaa  
 1 5 10 15

Glu Ser Cys Xaa  
 20

&lt;210&gt; 157

&lt;211&gt; 66

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 157

Met Ser Leu Thr Tyr Ser Trp Lys Lys Ser Lys Val Thr Lys Phe Asn  
 1 5 10 15

Leu Ser Thr Leu Arg Met Thr Val Thr Asn Lys Asn Arg Thr Val Gln  
 20 25 30

Lys Cys Ala Lys Asp Thr Arg Lys Leu Asn Asn Ile Asn Ser Met Ile  
 35 40 45

Ile Val Ile Leu Tyr Thr Met Glu Ser Lys Gln Ile Phe Phe His Gly  
 50 55 60

Asn Ser  
 65

&lt;210&gt; 158

&lt;211&gt; 41

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 158

Met Met Thr Gly Glu Ala Arg Glu Ser Gln Ile Ala Leu Tyr Lys Gln  
 1 5 10 15

Arg Phe Arg Glu Phe Arg Glu Glu Gly Arg Thr Ile Tyr Lys Gly Arg  
 20 25 30

Trp Lys Arg Ser His Leu Ala Glu Gly  
 35 40

&lt;210&gt; 159

&lt;211&gt; 31

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; UNSURE

&lt;222&gt; (7)

&lt;400&gt; 159

Met Leu Glu Leu Gly Leu Xaa Pro Lys Leu Thr Ser Glu Tyr Arg Phe  
 1 5 10 15

63

Pro Pro Asn Cys Met Ile Leu His Ile Trp Ser Gln Leu Glu Val  
                   20                  25                  30

<210> 160  
 <211> 75  
 <212> PRT  
 <213> Homo sapiens

<400> 160  
 Met Tyr Ile Tyr Ile Cys His His Phe Lys Asn Gln Ala Phe Lys Val  
       1                  5                  10                  15

Lys Leu Ser Phe Pro His Ile Phe Phe His Ser Ile Phe Tyr Gln Tyr  
                   20                  25                  30

Arg His Ser Leu Leu Leu Leu Ser Phe Gln Phe Pro Ile Ile Ser Ser  
                   35                  40                  45

His Pro Ile Phe Cys Ala Ser Ser Val Phe Lys Thr His Ser Pro Ser  
           50                  55                  60

Ala Ala Met Ala Pro Thr Ile Ile Phe Ile Thr  
       65                  70                  75

<210> 161  
 <211> 36  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> UNSURE  
 <222> (7) .. (13)

<400> 161  
 Met Lys Arg Gly Asn Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Thr Pro  
       1                  5                  10                  15

Cys Lys Asp Trp Ser His Thr Ala Met Ser Gln Glu Pro Pro Val Leu  
                   20                  25                  30

Val Arg Val Leu  
           35

<210> 162  
 <211> 24  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> UNSURE  
 <222> (9)

<220>  
 <221> UNSURE  
 <222> (20)

<400> 162

Met Trp Ala Ala Trp Arg Arg Arg Xaa Asn Gly Phe Phe Pro Arg Ile  
1 5 10 15

Pro Gly Lys Xaa Arg Gly Pro Asn  
20

<210> 163

<211> 31

<212> PRT

<213> Homo sapiens

<400> 163

Met Cys His Ser Leu Tyr Arg Phe Leu Asn Cys His Ser Arg Tyr Tyr  
1 5 10 15

Ile Val Tyr Thr Tyr Leu Thr Ile Phe Tyr Trp Cys His His Phe  
20 25 30

<210> 164

<211> 134

<212> PRT

<213> Homo sapiens

 $\langle 220 \rangle$ 

<221> UNSURE

 $\langle 222 \rangle \quad (2) \dots (22)$ 

**<220>**

<221> UNSURE

<222> (39) .. (67)

<220>

<221> UNSURE

 $\langle 222 \rangle \quad (79) \dots (113)$ 

<400> 164

Met Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Ala Gly Lys Arg Glu Asn Gln Lys Asp Ser  
20 25 30

Ser Val Arg Arg Thr Trp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
50 55 60

Xaa Xaa Xaa Arg Phe Ser Pro Arg Ala Tyr Arg Lys Lys Val Xaa Xaa  
65 70 75 80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
85 90 95

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
            100                        105                        110

65

Xaa Arg His Asn Arg Lys Leu Ile His Leu Ser Ser Lys Phe Leu Ile  
                   115                                  120                                  125

Ile Asn Val Ile Ala Ser  
                   130

&lt;210&gt; 165

&lt;211&gt; 51

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 165

Met Ser Lys Val Asp Leu Phe Ile Thr Asp Ser Phe Lys Lys Phe Asn  
       1                                  5                                  10                                  15

Gln Tyr Leu Leu Ala Thr Tyr Ser Thr Ser Gly Thr Gln Gly Ile Trp  
                                   20                                  25                                  30

Ser Thr Thr Met Lys Lys Arg Asp Trp Thr Leu Lys Glu His Arg Ser  
                   35                                  40                                  45

Cys His Phe  
           50

&lt;210&gt; 166

&lt;211&gt; 60

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 166

Met Ser Asp Ser Arg Leu Cys Ser Cys Phe Leu His Thr Leu Ile Phe  
       1                                  5                                  10                                  15

Leu Asn Ile Ser Lys Ile Gln Ser Gly Ser Lys Ile Thr Cys Lys Asn  
                                   20                                  25                                  30

Ile Leu Ala Gln Glu Phe Asp Arg Leu Lys Ile Asn Tyr Leu Lys Tyr  
                   35                                  40                                  45

Ile Lys Gln Glu Val Tyr Leu Leu Tyr Ser Met Tyr  
           50                                  55                                  60

&lt;210&gt; 167

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 167

Met Val Phe Gln Lys Thr Ser Leu Tyr Ser Asn Asn Ile Leu Leu  
       1                                  5                                  10                                  15

&lt;210&gt; 168

&lt;211&gt; 106

&lt;212&gt; PRT

66

&lt;213&gt; Homo sapiens

&lt;400&gt; 168

Cys Pro Ala Ala Tyr Thr Val Phe Leu Thr Arg Ile Ile Val Lys Tyr  
 1 5 10 15

Tyr Leu Asn Arg Gly Leu Phe Ser Glu Thr Pro Ser Asn Leu Lys Val  
 20 25 30

Glu Glu Lys Gly Trp Val Trp Trp Leu Met Pro Val Thr Pro Ala Leu  
 35 40 45

Trp Glu Ala Glu Ala Gly Gly Ser Leu Glu Leu Ser Leu Arg Pro Gly  
 50 55 60

Trp Ala Thr Pro Ser Leu Pro Lys Asn Thr Lys Met Ser Gln Ala Trp  
 65 70 75 80

Trp Cys Thr Pro Val Val Pro Ala Ala Leu Gly Ala Glu Val Gly Gly  
 85 90 95

Arg Leu Gly Pro Arg Arg Trp Arg Leu Gln  
 100 105

&lt;210&gt; 169

&lt;211&gt; 19

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 169

Met Gly Pro Asp Arg Leu Lys Gln Lys Ser Asn Thr Ala Val Val Ser  
 1 5 10 15

Arg Trp Ile

&lt;210&gt; 170

&lt;211&gt; 47

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; UNSURE

&lt;222&gt; (3) .. (4)

&lt;220&gt;

&lt;221&gt; UNSURE

&lt;222&gt; (13)

&lt;220&gt;

&lt;221&gt; UNSURE

&lt;222&gt; (16)

&lt;400&gt; 170

Met Asp Xaa Xaa Lys Trp Arg Met Arg Arg Gln Pro Xaa Ile Asn Xaa  
 1 5 10 15

67

Met Tyr Gln Thr Val Thr Ile Cys Glu Glu Tyr Cys Val Tyr Thr Asn  
                             20                            25                            30

Arg Lys Gln Leu Lys Ala Phe Asn Met Cys Gly Trp Gly Glu Arg  
                             35                            40                            45

&lt;210&gt; 171

&lt;211&gt; 197

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 171

Gln Glu Ala Gln Ile Met Lys Lys Leu Arg His Asp Lys Leu Val Pro  
           1                            5                            10                            15

Leu Tyr Ala Val Val Ser Glu Glu Pro Ile Tyr Ile Val Thr Glu Phe  
                             20                            25                            30

Met Ser Lys Gly Ala Tyr Ser Leu Ser Ile Arg Asp Trp Asp Glu Ile  
                             35                            40                            45

Arg Gly Asp Asn Val Lys His Tyr Lys Ile Arg Lys Leu Asp Asn Gly  
                             50                            55                            60

Gly Tyr Tyr Ile Thr Thr Arg Ala Gln Phe Asp Thr Leu Gln Lys Leu  
           65                            70                            75                            80

Val Lys His Tyr Thr Glu His Ala Asp Gly Leu Cys His Lys Leu Thr  
                             85                            90                            95

Thr Val Cys Pro Thr Val Lys Pro Gln Thr Gln Gly Leu Ala Lys Asp  
                             100                            105                            110

Ala Trp Glu Ile Pro Arg Glu Ser Leu Arg Leu Glu Val Lys Leu Gly  
                             115                            120                            125

Gln Gly Cys Phe Gly Glu Val Trp Met Gly Thr Trp Asn Gly Thr Thr  
           130                            135                            140

Lys Val Ala Ile Lys Thr Leu Lys Pro Gly Thr Met Met Pro Glu Ala  
           145                            150                            155                            160

Phe Leu Gln Glu Ala Gln Ile Met Lys Lys Leu Arg His Asp Lys Leu  
                             165                            170                            175

Val Pro Leu Tyr Ala Val Val Ser Glu Glu Pro Ile Tyr Ile Val Thr  
                             180                            185                            190

Glu Phe Met Ser Lys  
                             195

&lt;210&gt; 172

&lt;211&gt; 59

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;



68

&lt;221&gt; UNSURE

&lt;222&gt; (28) .. (49)

&lt;400&gt; 172

Met Cys Ile Met His Ile Asn Thr Phe Asn Leu Cys Asn His Leu Met  
 1 5 10 15

Arg Trp Leu Leu Leu Lys Ser Pro Leu Cys Thr Xaa Xaa Xaa Xaa Xaa  
 20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 35 40 45

Xaa Gln Lys Pro Lys Pro Thr Val His Gly Ile  
 50 55

&lt;210&gt; 173

&lt;211&gt; 56

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; UNSURE

&lt;222&gt; (14) .. (21)

&lt;400&gt; 173

Met Lys Pro Ile Arg Gln Leu Val Pro Phe Thr Leu Glu Xaa Xaa Xaa  
 1 5 10 15

Xaa Xaa Xaa Xaa Xaa Leu Tyr Leu Glu His Leu Thr Cys Arg Lys Arg  
 20 25 30

Arg Gly Lys Thr Phe Leu Gly Lys Arg Lys Ala Val Ala Val Pro Lys  
 35 40 45

Ser Lys His Phe Trp Gln Gly Phe  
 50 55

&lt;210&gt; 174

&lt;211&gt; 104

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 174

Met Leu Lys His Leu Gln Val Leu Asp Leu His Gln Cys Ser Leu Thr  
 1 5 10 15

Ala Asp Asp Val Met Ser Leu Thr Gln Val Ile Pro Leu Leu Ser Asn  
 20 25 30

Leu Gln Glu Leu Asp Leu Ser Ala Asn Lys Lys Met Gly Ser Ser Ser  
 35 40 45

Glu Asn Leu Leu Ser Arg Leu Arg Phe Leu Pro Ala Leu Lys Ser Leu  
 50 55 60

Val Ile Asn Asn Cys Ala Leu Glu Ser Glu Thr Phe Thr Ala Leu Ala

69

65		70		75		80									
Glu	Ala	Ser	Val	His	Leu	Ser	Ala	Leu	Glu	Val	Phe	Asn	Leu	Ser	Trp
				85					90					95	

Glu	Gln	Val	Cys	Trp	Trp	Ala	Thr
							100

&lt;210&gt; 175

&lt;211&gt; 490

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 175

Met	Ser	Gln	Thr	Arg	Lys	Lys	Thr	Ser	Ser	Glu	Gly	Glu	Thr	Lys	Pro
1				5					10					15	

Gln	Thr	Ser	Thr	Val	Asn	Lys	Phe	Leu	Arg	Gly	Ser	Asn	Ala	Glu	Ser
			20					25					30		

Arg	Lys	Glu	Asp	Asn	Asp	Leu	Lys	Thr	Ser	Asp	Ser	Gln	Pro	Ser	Asp
	35						40					45			

Trp	Ile	Gln	Lys	Thr	Ala	Thr	Ser	Glu	Thr	Ala	Lys	Pro	Leu	Ser	Ser
	50					55					60				

Glu	Met	Glu	Trp	Arg	Ser	Ser	Met	Glu	Lys	Asn	Glu	His	Phe	Leu	Gln
65					70					75					80

Lys	Leu	Gly	Lys	Lys	Ala	Val	Asn	Lys	Cys	Leu	Asp	Leu	Asn	Asn	Cys
				85					90					95	

Gly	Leu	Thr	Thr	Ala	Asp	Met	Lys	Glu	Met	Gly	Glu	Ala	Phe	Glu	Met
			100					105					110		

Ile	Pro	Glu	Leu	Glu	Glu	Leu	Asn	Leu	Ser	Trp	Asn	Ser	Lys	Val	Gly
		115					120					125			

Gly	Asn	Leu	Pro	Leu	Ile	Leu	Gln	Lys	Phe	Gln	Lys	Gly	Ser	Lys	Ile
	130					135					140				

Gln	Met	Ile	Glu	Leu	Val	Ala	Cys	Ser	Leu	Thr	Ser	Glu	Asp	Gly	Thr
145					150					155					160

Phe	Leu	Gly	Gln	Leu	Leu	Pro	Met	Leu	Gln	Ser	Leu	Glu	Val	Leu	Asp
				165					170					175	

Leu	Ser	Ile	Asn	Arg	Asp	Ile	Val	Gly	Ser	Leu	Asn	Ser	Ile	Ala	Gln
			180					185					190		

Gly	Leu	Lys	Ser	Thr	Ser	Asn	Leu	Lys	Val	Leu	Lys	Leu	His	Ser	Cys
		195					200					205			

Gly	Leu	Ser	Gln	Lys	Ser	Val	Lys	Ile	Leu	Asp	Ala	Ala	Phe	Arg	Tyr
	210					215					220				

Leu	Gly	Glu	Leu	Arg	Lys	Leu	Asp	Leu	Ser	Cys	Asn	Lys	Asp	Leu	Gly
225					230					235				240	

70

Gly Gly Phe Glu Asp Ser Pro Ala Gln Leu Val Met Leu Lys His Leu  
 245 250 255  
 Gln Val Leu Asp Leu His Gln Cys Ser Leu Thr Ala Asp Asp Val Met  
 260 265 270  
 Ser Leu Thr Gln Val Ile Pro Leu Leu Ser Asn Leu Gln Glu Leu Asp  
 275 280 285  
 Leu Ser Ala Asn Lys Lys Met Gly Ser Ser Ser Glu Asn Leu Leu Ser  
 290 295 300  
 Arg Leu Arg Phe Leu Pro Ala Leu Lys Ser Leu Val Ile Asn Asn Cys  
 305 310 315 320  
 Ala Leu Glu Ser Glu Thr Phe Thr Ala Leu Ala Glu Ala Ser Val His  
 325 330 335  
 Leu Ser Ala Leu Glu Val Phe Asn Leu Ser Trp Asn Lys Cys Val Gly  
 340 345 350  
 Gly Asn Leu Lys Leu Leu Leu Glu Thr Leu Lys Leu Ser Met Ser Leu  
 355 360 365  
 Gln Val Leu Arg Leu Ser Ser Cys Ser Leu Val Thr Glu Asp Val Ala  
 370 375 380  
 Leu Leu Ala Ser Val Ile Gln Thr Gly His Leu Ala Lys Leu Gln Lys  
 385 390 395 400  
 Leu Asp Leu Ser Tyr Asn Asp Ser Ile Cys Asp Ala Gly Trp Thr Met  
 405 410 415  
 Phe Cys Gln Asn Val Arg Phe Leu Lys Glu Leu Ile Glu Leu Asp Ile  
 420 425 430  
 Ser Leu Arg Pro Ser Asn Phe Arg Asp Cys Gly Gln Trp Phe Arg His  
 435 440 445  
 Leu Leu Tyr Ala Val Thr Lys Leu Pro Gln Ile Thr Glu Ile Gly Met  
 450 455 460  
 Lys Arg Trp Ile Leu Pro Ala Ser Gln Glu Glu Glu Leu Glu Cys Phe  
 465 470 475 480  
 Asp Gln Asp Lys Lys Lys Lys His Ser Leu  
 485 490

&lt;210&gt; 176

&lt;211&gt; 136

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 176

Met His Leu Leu Ser Asp Gly Lys Glu Gly Ser Thr Tyr Lys Pro Phe  
 1 5 10 15

71

Gln Glu Ile Ser Ser Ser Ser Lys Ser Gly Arg Lys Gly Ser Lys Ala  
                     20                    25                    30  
 Thr Ile Ser Phe Met Ser Ala Val Val Asn Pro Gln Leu Phe Lys Ser  
                     35                    40                    45  
 Arg His Leu Leu Thr Ala Phe Leu Pro Ser Phe Cys Arg Lys Cys Ser  
                     50                    55                    60  
 Phe Phe Ser Ile Leu Asp Leu His Ser Ile Ser Glu Leu Arg Gly Leu  
                     65                    70                    75                    80  
 Ala Val Ser Glu Val Ala Val Phe Cys Ile Gln Ser Leu Gly Trp Glu  
                     85                    90                    95  
 Ser Leu Val Leu Arg Ser Leu Ser Ser Phe Leu Leu Ser Ala Leu Glu  
                     100                    105                    110  
 Pro Leu Arg Asn Leu Leu Thr Val Glu Val Trp Gly Leu Val Ser Pro  
                     115                    120                    125  
 Ser Glu Glu Val Phe Phe Leu Val  
                     130                    135

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